expressed in the heart and skeletal muscle, and can be useful in promoting wound healing in this tissue, or exhibit anti-bacterial or anti-viral effects. Moreover, zmse1, its agonists or antagonists can be useful in treatment of inflammatory heart or cardiovascular conditions, muscle inflammation, inflammation during and after surgery, arthritis, asthma, inflammatory bowel disease, diverticulitis, and the like. Moreover, direct measurement of zmse1 polypeptide and anti-zmse1 antibodies can be useful in diagnosing inflammatory diseases such as reperfusion ischemia, inflammatory bowel disease, diverticulitis, asthma, pelvic inflammatory disease (PID), psoriasis, arthritis, melanoma, and other inflammatory diseases. Moreover zmse1 antagonists can be useful in treatment of myocarditis, atherosclerosis, pelvic inflammatory disease, (PID), psoriasis, arthritis, eczema, scleroderma, and other inflammatory diseases.

As such, zmse1 polypeptide, agonists or its antagonists, have potential uses in inflammatory diseases such as asthma and arthritis. For example, if zmse1 is proinflammatory, antagonists would be valuable in asthma therapy or other anti-inflammatory therapies where migration of lymphocytes is damaging. In addition, zmse1 can serve other important roles in lung function, for instance, bronchodilation, tissue elasticity, recruitment of lymphocytes in lung infection and damage. Assays to assess the activity of zmse1 in lung cells are discussed in Laberge, S. et al., Am. J. Respir. Cell Mol. Biol. 17:193-202, 1997; Rumsseng, V. et al., J. Immunol., 159:2904-2910, 1997; and Schluesener, H.J. et al., J. Neurosci. Res. 44:606-611, 1996. Methods to determine proinflammatory and antiinflammatory qualities of zmse1 its agonists or its antagonists are known in the art. Moreover, other molecular biological, immunological, and biochemical techniques known in the art and disclosed herein can be used to determine zmse1 activity and to isolate agonists and antagonists.

The activity of molecules of the present invention may be measured using a variety of assays that, for example, measure neogenesis or hyperplasia (i.e., proliferation) of cardiac or other cells based on the potential effects of activity of zmse1 in those tissues. Additional activities likely associated with the polypeptides of the present invention include proliferation of endothelial cells, cardiomyocytes, fibroblasts, skeletal myocytes directly or indirectly through other growth factors; action as a

chemotaxic factor for endothelial cells, fibroblasts and/or phagocytic cells; osteogenic factor; and factor for expanding mesenchymal stem cell and precursor populations.

Proliferation can be measured *in vitro* using cultured cells or *in vivo* by administering molecules of the present invention to the appropriate animal model. Generally, proliferative effects are seen as an increase in cell number, and may include inhibition of apoptosis as well as stimulation of mitogenesis. Cultured cells for use in these assays include cardiac fibroblasts, cardiac myocytes, skeletal myocytes, and human umbilical vein endothelial cells from primary cultures, among other cell types. Suitable established cell lines include: NIH 3T3 fibroblasts (ATCC No. CRL-1658), CHH-1 chum heart cells (ATCC No. CRL-1680), H9c2 rat heart myoblasts (ATCC No. CRL-1446), Shionogi mammary carcinoma cells (Tanaka et al., Proc. Natl. Acad. Sci. 89:8928-8932, 1992), and LNCap.FGC adenocarcinoma cells (ATCC No. CRL-1740.) Assays measuring cell proliferation are well known in the art. For example, assays measuring proliferation include such assays as chemosensitivity to neutral red dye (Cavanaugh et al., Investigational New Drugs 8:347-354, 1990), incorporation of radiolabeled nucleotides (Cook et al., Analytical Biochem. 179:1-7, 1989), incorporation of 5-bromo-2'-deoxyuridine (BrdU) in the DNA of proliferating cells (Porstmann et al., J. Immunol. Methods 82:169-179, 1985), and use of tetrazolium salts (Mosmann, J. Immunol. Methods 65:55-63, 1983; Alley et al., Cancer Res. 48:589-601, 1988; Marshall et al., Growth Reg. 5:69-84, 1995; and Scudiero et al., Cancer Res. 48:4827-4833, 1988).

Differentiation is a progressive and dynamic process, beginning with pluripotent stem cells and ending with terminally differentiated cells. Pluripotent stem cells that can regenerate without commitment to a lineage express a set of differentiation markers that are lost when commitment to a cell lineage is made. Progenitor cells express a set of differentiation markers that may or may not continue to be expressed as the cells progress down the cell lineage pathway toward maturation. Differentiation markers that are expressed exclusively by mature cells are usually functional properties such as cell products, enzymes to produce cell products, and receptors. The stage of a cell population's differentiation is monitored by identification of markers present in the cell population. Myocytes, osteoblasts, adipocytes,

chrondrocytes, fibroblasts and reticular cells are believed to originate from a common mesenchymal stem cell (Owen et al., Ciba Fdn. Symp. 136:42-46, 1988). Markers for mesenchymal stem cells have not been well identified (Owen et al., J. of Cell Sci. 87:731-738, 1987), so identification is usually made at the progenitor and mature cell stages. The novel polypeptides of the present invention may be useful for studies to isolate mesenchymal stem cells and myocyte or other progenitor cells, both *in vivo* and *ex vivo*.

There is evidence to suggest that factors that stimulate specific cell types down a pathway towards terminal differentiation or dedifferentiation affect the entire cell population originating from a common precursor or stem cell. Thus, the present invention includes stimulating or inhibiting the proliferation of myocytes, smooth muscle cells, osteoblasts, adipocytes, chrondrocytes and endothelial cells. Molecules of the present invention for example, may while stimulating proliferation or differentiation of cardiac myocytes, inhibit proliferation or differentiation of adipocytes, by virtue of the affect on their common precursor/stem cells. Thus molecules of the present invention may have use in inhibiting chondrosarcomas, atherosclerosis, restenosis and obesity.

Assays measuring differentiation include, for example, measuring cell markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, FASEB, 5:281-284, 1991; Francis, Differentiation 57:63-75, 1994; Raes, Adv. Anim. Cell Biol. Technol. Bioprocesses, 161-171, 1989; all incorporated herein by reference). Alternatively, zmse1 polypeptide itself can serve as an additional cell-surface marker associated with stage-specific expression of a tissue. As such, direct measurement of zmse1 polypeptide, or its loss of expression in a tissue as it differentiates, can serve as a marker for differentiation of tissues.

Similarly, direct measurement of zmse1 polypeptide, or its loss of expression in a tissue can be determined in a tissue or cells as they undergo tumor progression. As the Ras and Rho family, and their effectors are involved with increases in invasiveness and motility of cells, the gain or loss of expression of zmes1 in a pre-cancerous or cancerous condition, in comparison to normal tissue, can serve as a

diagnostic for transformation, invasion and metastasis in tumor progression. As such, knowledge of a tumor's stage of progression or metastasis will aid the physician in choosing the most proper therapy, or aggressiveness of treatment, for a given individual cancer patient. Methods of measuring gain and loss of expression (of either mRNA or protein) are well known in the art and described herein and can be applied to zmse1 expression. For example, appearance or disappearance of polypeptides that regulate cell motility can be used to aid diagnosis and prognosis of prostate cancer (Banyard, J. and Zetter, B.R., Cancer and Metast. Rev. 17:449-458, 1999). As an effector of cell motility, zmse1 gain or loss of expression may serve as a diagnostic for prostate and other cancers.

Zmse1 can also be used to identify inhibitors (antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of zmse1. In addition to those assays disclosed herein, samples can be tested for inhibition of zmse1 activity within a variety of assays designed to measure receptor binding or the stimulation/inhibition of zmse1-dependent cellular responses. For example, zmse1-expressing cell lines can be transfected with a reporter gene construct that is responsive to a zmse1-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a zmse1-DNA response element operably linked to a gene encoding an assayable protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., Proc. Natl. Acad. Sci. USA 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. Cell 56: 563-72, 1989). Cyclic AMP response elements are reviewed in Roestler et al., J. Biol. Chem. 263:9063-6; 1988 and Habener, Molec. Endocrinol. 4:1087-94; 1990. Hormone response elements are reviewed in Beato, Cell 56:335-44; 1989. Candidate compounds, solutions, mixtures or extracts are tested for the ability to inhibit the activity of zmse1 on the target cells as evidenced by a decrease in zmse1 stimulation of reporter gene expression. Assays of this type will detect compounds that directly block effectors that bind zmse1 (or proteins to which zmse1 is an effector), as well as compounds that block processes in the cellular pathway upstream or subsequent to receptor-ligand binding. In the alternative, compounds or

other samples can be tested for direct blocking of zmsel binding to receptor using zmsel tagged with a detectable label (e.g., ^{125}I, biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a test sample to inhibit the binding of labeled zmsel to the receptor is indicative of inhibitory activity, which can be confirmed through secondary assays. Receptors used within binding assays may be cellular receptors or isolated, immobilized receptors.

A role for zmsel in the induction of cell motility suggests a role in spermatogenesis, a process that is remarkably similar to the development of blood cells (hematopoiesis). Briefly, spermatogonia undergo a maturation process similar to the differentiation of hematopoietic stem cells. Moreover, in view of cell motility effects, zmsel polypeptides, agonists and antagonists have enormous potential in both *in vitro* and *in vivo* applications. For example, cell motility and polypeptides associated therewith have been implicated as a critical determinants in prostate cancer metastasis (Banyard, J. and Zetter, B.R., Cancer and Metast. Rev. 17:449-458, 1999). As an effector of cell motility, zmsel may serve as a diagnostic for such cancers, and zmsel polypeptides, agonists and antagonists have therapeutic potential to treat such diseases. Zmsel polypeptides, agonists and antagonists may also prove useful in modulating spermatogenesis and thus aid in overcoming infertility, or as therapeutics or diagnostics for male reproductive cancers such as prostate and testicular cancers. Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, zmsel polypeptides, agonists or antagonists may find application in the treatment of male infertility, reproductive cancers, or as a male contraceptive agents.

The zmsel polypeptides, antagonists of agonists, of the present invention can also modulate sperm capacitation. Before reaching the oocyte or egg and initiating an egg-sperm interaction, the sperm must be activated. The sperm undergo a gradual capacitation, lasting up to 3 or 4 hours *in vitro*, during which the plasma membrane of the sperm head and the outer acrosomal membrane fuse to form vesicles that facilitate the release of acrosomal enzymes. The acrosomal membrane surrounds the acrosome or acrosomal cap which is located at the anterior end of the nucleus in the sperm head. In order for the sperm to fertilize egg the sperm must penetrate the oocyte.

To enable this process the sperm must undergo acrosomal exocytosis, also known as the acrosomal reaction, and release the acrosomal enzymes in the vicinity of the oocyte. These enzymes enable the sperm to penetrate the various oocyte layers, (the cumulus oophorus, the corona radiata and the zona pellucida). The released acrosomal enzymes include hyaluronidase and proacrosin, in addition to other enzymes such as proteases. During the acrosomal reaction, proacrosin is converted to acrosin, the active form of the enzyme, which is required for and must occur before binding and penetration of the zona pellucida is possible. A combination of the acrosomal lytic enzymes and sperm tail movements allow the sperm to penetrate the oocyte layers. Numerous sperm must reach the egg and release acrosomal enzymes before the egg can finally be fertilized. Only one sperm will successfully bind to, penetrate and fertilize the egg, after which the zona hardens so that no other sperm can penetrate the egg (Zaneveld, in Male Infertility Chapter 11, Comhaire (Ed.), Chapman & Hall, London, 1996). Peptide hormones, such as insulin homologs are associated with sperm activation and egg-sperm interaction. For instance, capacitated sperm incubated with relaxin show an increased percentage of progressively motile sperm, increased zona penetration rates, and increased percentage of viable acrosome-reacted sperm (Carrell et al., Endocr. Res. 21:697-707, 1995). Similarity of the zmse1 polypeptide structure to signal transduction molecules and the potential of zmse1 effects on indirectly effecting cell-cell interaction in the testis, prostate and uterus suggests that the zmse1 polypeptides described herein play a role in these and other reproductive processes.

Accordingly, proteins of the present invention can have applications in enhancing fertilization during assisted reproduction in humans and in animals. Such assisted reproduction methods are known in the art and include artificial insemination, *in vitro* fertilization, embryo transfer and gamete intrafallopian transfer. Such methods are useful for assisting men and women who have physiological or metabolic disorders preventing natural conception or can be used to enhance *in vitro* fertilization. Such methods are also used in animal breeding programs, such as for livestock breeding and could be used as methods for the creation of transgenic animals. Proteins of the present invention can be combined with sperm, an egg or an egg-sperm mixture prior to fertilization of the egg. In some species, sperm capacitate spontaneously during *in vitro*

fertilization procedures, but normally sperm capacitate over an extended period of time both *in vivo* and *in vitro*. It is advantageous to increase sperm activation during such procedures to enhance the likelihood of successful fertilization. The washed sperm or sperm removed from the seminal plasma used in such assisted reproduction methods has been shown to have altered reproductive functions, in particular, reduced motility and zona interaction. To enhance fertilization during assisted reproduction methods sperm is capacitated using exogenously added compounds. Suspension of the sperm in seminal plasma from normal subjects or in a "capacitation media" containing a cocktail of compounds known to activate sperm, such as caffeine, dibutyl cyclic adenosine monophosphate (dbcAMP) or theophylline, have resulted in improved reproductive function of the sperm, in particular, sperm motility and zonae penetration (Park et al., Am. J. Obstet. Gynecol. 158:974-9, 1988; Vandevoort et al., Mol. Repro. Develop. 37:299-304, 1993; Vandevoort and Overstreet, J. Androl. 16:327-33, 1995). The presence of immunoreactive relaxin *in vivo* and in association with cryopreserved semen, was shown to significantly increase sperm motility (Juang et al., Anim. Reprod. Sci. 20:21-9, 1989; Juang et al., Anim. Reprod. Sci. 22:47-53, 1990). Porcine relaxin stimulated sperm motility in cryopreserved human sperm (Colon et al., Fertil. Steril. 46:1133-39, 1986; Lessing et al., Fertil. Steril. 44:406-9, 1985) and preserved ability of washed human sperm to penetrate cervical mucus *in vitro* (Brenner et al., Fertil. Steril. 42:92-6, 1984). Polypeptides of the present invention can used in such methods to enhance viability of cryopreserved sperm, enhance sperm motility and enhance fertilization, particularly in association with methods of assisted reproduction.

A zmse1 polypeptide can be expressed as a fusion with an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains two constant region domains and lacks the variable region. Methods for preparing such fusions are disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two non-Ig polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify ligand or binding partners, as an *in vitro* assay tool, or a zmse1 ligand antagonist. For use in assays, the chimeras are bound to a support via the F_c region and used in an ELISA format.

A zmse1 polypeptide can also be used for purification of ligand, biomolecular substrates, or other proteins or antibodies that bind it. The zmse1 polypeptide or a polypeptide fragment containing the zmse1 CRIB motif can be used. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand, cell lysates, membrane preparations, or lipid preparations, are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/ anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Zmse1 polypeptides can also be used to prepare antibodies that bind to zmse1 epitopes, peptides or polypeptides. The zmse1 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. One of skill in the art would recognize that antigenic, epitope-bearing polypeptides contain a sequence of at least 6, preferably at least 9, and more preferably at least 15 to about 30 contiguous amino acid residues of a zmse1 polypeptide (e.g., SEQ ID NO:2). Polypeptides comprising a larger portion of a zmse1 polypeptide, i.e., from 30 to 10 residues up to the entire length of the amino acid sequence are included. Antigens or immunogenic epitopes can also include attached tags, adjuvants and carriers, as described herein. Suitable antigens include the zmse1 polypeptide encoded by SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 356 (Val), or a contiguous 13 to 343 amino acid fragment thereof. Other suitable antigens include the CRIB motif, N-terminal domain, variable C-terminal domain, and C-terminal tail as disclosed herein. Preferred peptides to use as antigens are hydrophilic peptides such as those predicted by one of skill in the art from a hydrophobicity plot, determined, for example, from a Hopp/Woods hydrophilicity profile based on a sliding six-residue window, with buried G, S, and T residues and exposed H, Y, and W residues ignored (See, Figure 1). Zmse1 hydrophilic peptides include peptides comprising amino acid sequences selected from the group consisting of: (1) amino acid number 96 (Glu) to amino acid number 101 (Asp) of SEQ ID NO:2; (2) amino acid number 226 (Asp) to amino acid number 231 (Asp) of SEQ ID NO:2; (3) amino acid number 346 (Met) to amino acid number 351 (Glu) of SEQ ID NO:2; (4) amino acid number 347 (Asp) to amino acid number 352 (Asp) of SEQ ID NO:2; and (5) amino acid number 348 (Glu) to amino acid number 353 (Glu) of SEQ ID NO:2. Antibodies from an immune response generated by inoculation of an animal with these antigens can be isolated and

purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, Current Protocols in Immunology, Coligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a zmse1 polypeptide or a fragment thereof. The immunogenicity of a zmse1 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zmse1 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for

adverse immune reactions upon administration to humans is reduced. Moreover, human antibodies can be produced in transgenic, non-human animals that have been engineered to contain human immunoglobulin genes as disclosed in WIPO Publication WO 98/24893. It is preferred that the endogenous immunoglobulin genes in these animals be inactivated or eliminated, such as by homologous recombination.

Antibodies are considered to be specifically binding if: 1) they exhibit a threshold level of binding activity, and 2) they do not significantly cross-react with known related polypeptide molecules. A threshold level of binding is determined if anti-zmse1 antibodies herein bind to a zmse1 polypeptide, peptide or epitope with an affinity at least 10-fold greater than the binding affinity to control (non-zmse1) polypeptide. It is preferred that the antibodies exhibit a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-672, 1949).

Whether anti-zmse1 antibodies do not significantly cross-react with known related polypeptide molecules is shown, for example, by the antibody detecting zmse1 polypeptide but not known related polypeptides using a standard Western blot analysis (Ausubel et al., ibid.). Examples of known related polypeptides are those disclosed in the prior art, such as known orthologs, and paralogs, and similar known members of a protein family. Screening can also be done using non-human zmse1, and zmse1 mutant polypeptides. Moreover, antibodies can be "screened against" known related polypeptides, to isolate a population that specifically binds to the zmse1 polypeptides. For example, antibodies raised to zmse1 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to zmse1 will flow through the matrix under the proper buffer conditions. Screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to known closely related polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and

isolation of specific antibodies is well known in the art. See, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., Adv. in Immunol. 43: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J.W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984. Specifically binding anti-zmse1 antibodies can be detected by a number of methods in the art, and disclosed below.

A variety of assays known to those skilled in the art can be utilized to detect antibodies which bind to zmse1 proteins or polypeptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zmse1 protein or polypeptide.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to zmse1 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled zmse1 protein or peptide). Genes encoding polypeptides having potential zmse1 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB

Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the zmse1 sequences disclosed herein to identify proteins which bind to zmse1. These "binding polypeptides" which interact with zmse1 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding polypeptides can also be used in analytical methods such as for screening expression libraries and neutralizing activity, e.g., for blocking interaction between ligand and receptor. The binding polypeptides can also be used for diagnostic assays for determining circulating levels of zmse1 polypeptides; for detecting or quantitating soluble zmse1 polypeptides as marker of underlying pathology or disease. These binding polypeptides can also act as zmse1 "antagonists" to block zmse1 binding and signal transduction *in vitro* and *in vivo*. These anti-zmse1 binding polypeptides would be useful for inhibiting zmse1 activity or protein-binding.

Antibodies to zmse1 may be used for tagging cells that express zmse1; for isolating zmse1 by affinity purification; for diagnostic assays for determining circulating levels of zmse1 polypeptides; for detecting or quantitating soluble zmse1 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zmse1 activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to zmse1 or fragments thereof may be used *in vitro* to detect denatured zmse1 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Antibodies or polypeptides herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. For instance, polypeptides or antibodies of

the present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (receptor or antigen, respectively, for instance). More specifically, zmse1 polypeptides or anti-zmse1 antibodies, or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the anti-complementary molecule.

Suitable detectable molecules may be directly or indirectly attached to the polypeptide or antibody, and include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/ anticomplementary pair, where the other member is bound to the polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

In another embodiment, polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the polypeptide has multiple functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-

specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

In another embodiment, zmse1-cytokine fusion proteins or antibody-cytokine fusion proteins can be used for enhancing *in vivo* killing of target tissues (for example, blood, bone marrow or other cancers), if the zmse1 polypeptide or anti-zmse1 antibody targets the hyperproliferative blood or bone marrow cell (See, generally, Hornick et al., Blood 89:4437-47, 1997). Hornick et al. described fusion proteins that enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. Suitable zmse1 polypeptides or anti-zmse1 antibodies can target an undesirable cell or tissue (i.e., a tumor or a leukemia), and the fused cytokine mediate improved target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), for instance.

In yet another embodiment, if the zmse1 polypeptide or anti- zmse1 antibody targets vascular cells or tissues, such polypeptide or antibody may be conjugated with a radionuclide, and particularly with a beta-emitting radionuclide, to reduce restenosis. Such therapeutic approach poses less danger to clinicians who administer the radioactive therapy. For instance, iridium-192 impregnated ribbons placed into stented vessels of patients until the required radiation dose was delivered showed decreased tissue growth in the vessel and greater luminal diameter than the control group, which received placebo ribbons. Further, revascularisation and stent thrombosis were significantly lower in the treatment group. Similar results are predicted with targeting of a bioactive conjugate containing a radionuclide, as described herein.

The bioactive polypeptide or antibody conjugates described herein can be delivered intravenously, intraarterially or intraductally, or may be introduced locally at the intended site of action.

The polypeptides, antagonists, agonists, nucleic acid antibodies of the present invention can be used in treatment of disorders associated with cancer, metastasis, vasoconstriction, heart arrhythmia, heart inflammation, congestive heart disease, muscle spasms and fatigue, inflammation, testicular function, fertility, birth

control, and the like. The molecules of the present invention can be used to modulate contractility or inflammation or to treat or prevent development of pathological conditions in diverse tissues. In particular, certain syndromes/diseases may be amenable to such diagnosis, treatment or prevention.

Diagnostic methods of the present invention involve the detection of zmse1 polypeptides in the serum or tissue biopsy of a patient undergoing analysis of heart, spleen, testicular or muscle function or evaluation for possible cancers. Such polypeptides can be detected using immunoassay techniques and antibodies, described herein, that are capable of recognizing polypeptide epitopes. More specifically, the present invention contemplates methods for detecting zmse1 polypeptides comprising:

exposing a test sample potentially containing zmse1 polypeptides to an antibody attached to a solid support, wherein said antibody binds to a first epitope of a zmse1 polypeptide;

washing the immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zmse1 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. Altered levels of zmse1 polypeptides in a test sample, such as serum sweat, saliva, biopsy, tumor biopsy, and the like, can be monitored as an indication of heart, spleen, testicular or muscle function or of cancer, invasion or metastasis or other disease, when compared against a normal control.

Additional methods using probes or primers derived, for example, from the nucleotide sequences disclosed herein can also be used to detect zmse1 expression in a patient sample, such as a blood, saliva, sweat, tissue sample, or the like. For example, probes can be hybridized to tumor tissues and the hybridized complex detected by *in situ* hybridization. Zmse1 sequences can also be detected by PCR amplification using cDNA generated by reverse translation of sample mRNA as a template (PCR Primer A Laboratory Manual, Dieffenbach and Dveksler, eds., Cold Spring Harbor Press, 1995). When compared with a normal control, both increases or

decreases of zmse1 expression in a patient sample, relative to that of a control, can be monitored and used as an indicator or diagnostic for disease.

Polynucleotides encoding zmse1 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zmse1 activity. For example, in disease states where cell migration or motility is impaired or deficient, introduction of a zmse1 gene could be used as a therapeutic. If a mammal has a mutated or absent zmse1 gene, the zmse1 gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a zmse1 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), retroviruses, papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

In another embodiment, a zmse1 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting

of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zmse1 gene transcription, such as to inhibit cell proliferation *in vivo*. Polynucleotides that are complementary to a segment of a zmse1-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NO:1) are designed to bind to zmse1-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zmse1 polypeptide-encoding genes in cell culture or in a subject.

The present invention also provides reagents which will find use in diagnostic applications. For example, the zmse1 gene, a probe comprising zmse1 DNA or RNA or a subsequence thereof can be used to determine if the zmse1 gene is present on chromosome 17 or if a mutation has occurred. Zmse1 is located at the 17q24.1 region of chromosome 17 (see, Example 3). Detectable chromosomal aberrations at the zmse1 gene locus include, but are not limited to, aneuploidy, gene copy number changes, translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing

PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995).

The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

The zmse1 gene is located at the 17q24.1 region of chromosome 17. Several genes of known function map to this region. Moreover, one of skill in the art would recognize that the 7q24 region is involved in several cancers, and that translocations, loss of heterogeneity (LOH) and other chromosomal abnormalities are often found in cancers. Thus, a marker in the 17q24.1 locus, such as provided by the polynucleotides of the present invention, would be useful in detecting translocations, aneuploidy, rearrangements, LOH other chromosomal abnormalities involving this region that are present in cancers. For example, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with the cancer susceptibility marker BRCA1, localized to 17q21, which is associated with breast, ovarian and prostate cancers (Hall, J.M. et al., Science 250:1684-1689, 1990). Zmse1 is localized to the 17q24.1, is likely a Rho family effector, and could also be directly involved in breast cancer or other tumors. Moreover, there is evidence for cancer resulting from mutations in the 17q24 region: the somatostatin receptor 2 gene (17q24) may be associated with cancers, such as small cell lung cancer (Zhang, C.-Y. et al., Biochem. Biophys. Res. Commun. 210:805-815, 1995); and esophageal cancers (Hennies, H.-C. et al., Genomics 29:537-540, 1995).

Moreover, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with pituitary and placental human growth hormone (GH), which maps to the 17q22-q24 region of chromosome 17. Mutations and deletions in the GH gene can create GH deficiencies and other diseases in humans, and such a diagnostic could assist physicians in determining the type of GH disease and

appropriate associated therapy. As such, use of inventive anti-zmse1 antibodies, polynucleotides, and polypeptides can be used for the detection of zmse1 polypeptide, mRNA or anti-zmse1 antibodies, thus serving as markers and be directly used for detecting or diagnosing growth hormone deficiencies or cancers using methods known in the art and described herein. For example, zmse1 can be used to detect abnormalities or genotypes associated with the cyclin-dependent kinase 3 (CDK3) gene, involved in controlling cell cycle and intracellular signaling, maps to 17q22-qter, and is likely involved in human cancer (Bullrich, F. et al., Cancer Res. 55:1199-1205, 1995). Moreover, Zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with dipeptidyl carboxypeptidase 1 (DCP1) (17q23), also known as Angiotensin I Converting Enzyme (ACE1), such as those that are implicated in heart disease, hypertension and male infertility (for example., see, Arbustini, E. et al., Brit. Heart J. 74:584-591, 1995; Cambien, F. et al., Nature 359:641-644, 1992; and Hagaman, J.R. et al., Proc. Natl. Acad. Sci. 95:2552-2557. 1998). Further, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 17q24 deletions and translocations associated with human diseases, such as in the myeloperoxidase locus (17q23.1), or in cancers. Moreover, amongst other genetic loci, those for myeloperoxidase deficiency, (17q23.1), loci associated with cataracts (17q24), defects in sodium channel voltage-gated type 2 (resulting in several different syndromes) (17q23.1-q25.3), all manifest themselves in human disease states as well as map to this region of the human genome. See the Online Mendelian Inheritance of Man (OMIM) gene map, and references therein, for this region of chromosome 17 on a publicly available WWW server (http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/getmap?chromosome=17q24.1). All of these serve as possible candidate genes for an inheritable disease which show linkage to the same chromosomal region as the zmse1 gene. Thus, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

Similarly, defects in the zmse1 gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid

in the detection, diagnosis prevention, and treatment associated with a zmse1 genetic defect.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling or diagnosing cancer. As such, the inventive anti-zmse1 antibodies, polynucleotides, and polypeptides can be used for the detection of zmse1 polypeptide, mRNA or anti-zmse1 antibodies, thus serving as markers and be directly used for detecting or genetic diseases or cancers, as described herein, using methods known in the art and described herein. Further, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 17q24.1 deletions and translocations associated with human diseases, such as those described above, or other translocations involved with malignant progression of tumors or other 17q24.1 mutations, which are expected to be involved in chromosome rearrangements in malignancy; or in other cancers. Similarly, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 17q24.1 trisomy and chromosome loss associated with human diseases or spontaneous abortion. Thus, zmse1 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

As discussed above, defects in the zmse1 gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a zmse1 genetic defect. In addition, zmse1 polynucleotide probes can be used to detect allelic differences between diseased or non-diseased individuals at the zmse1 chromosomal locus. As such, the zmse1 sequences can be used as diagnostics in forensic DNA profiling.

In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Analytical probes will be generally at least 20 nt in length, although somewhat shorter probes can be used (e.g., 14-17 nt). PCR primers are at least 5 nt in length, preferably 15 or more, more preferably 20-30 nt. For gross analysis of genes, or chromosomal DNA, a zmse1 polynucleotide probe may comprise an entire exon or more. Exons are readily

determined by one of skill in the art by comparing zmse1 sequences (SEQ ID NO:1) with the human genomic DNA for zmse1 (Genbank Accession No. AC026091). In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Most diagnostic methods comprise the steps of (a) obtaining a genetic sample from a potentially diseased patient, diseased patient or potential non-diseased carrier of a recessive disease allele; (b) producing a first reaction product by incubating the genetic sample with a zmse1 polynucleotide probe wherein the polynucleotide will hybridize to complementary polynucleotide sequence, such as in RFLP analysis or by incubating the genetic sample with sense and antisense primers in a PCR reaction under appropriate PCR reaction conditions; (iii) Visualizing the first reaction product by gel electrophoresis and/or other known method such as visualizing the first reaction product with a zmse1 polynucleotide probe wherein the polynucleotide will hybridize to the complementary polynucleotide sequence of the first reaction; and (iv) comparing the visualized first reaction product to a second control reaction product of a genetic sample from wild type patient. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the diseased or potentially diseased patient, or the presence of a heterozygous recessive carrier phenotype for a non-diseased patient, or the presence of a genetic defect in a tumor from a diseased patient, or the presence of a genetic abnormality in a fetus or pre-implantation embryo. For example, a difference in restriction fragment pattern, length of PCR products, length of repetitive sequences at the zmse1 genetic locus, and the like, are indicative of a genetic abnormality, genetic aberration, or allelic difference in comparison to the normal wild type control. Controls can be from unaffected family members, or unrelated individuals, depending on the test and availability of samples. Genetic samples for use within the present invention include genomic DNA, mRNA, and cDNA isolated form any tissue or other biological sample from a patient, such as but not limited to, blood, saliva, semen, embryonic cells, amniotic fluid, and the like. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Such methods of showing genetic linkage analysis to human disease phenotypes are well known in the art. For reference to PCR based methods in

diagnostics see see, generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998)).

Mutations associated with the zmse1 locus can be detected using nucleic acid molecules of the present invention by employing standard methods for direct mutation analysis, such as restriction fragment length polymorphism analysis, short tandem repeat analysis employing PCR techniques, amplification-refractory mutation system analysis, single-strand conformation polymorphism detection, RNase cleavage methods, denaturing gradient gel electrophoresis, fluorescence-assisted mismatch analysis, and other genetic analysis techniques known in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Marian, *Chest 108*:255 (1995), Coleman and Tsongalis, *Molecular Diagnostics* (Human Press, Inc. 1996), Elles (ed.) *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc. 1996), Landegren (ed.), *Laboratory Protocols for Mutation Detection* (Oxford University Press 1996), Birren *et al.* (eds.), *Genome Analysis, Vol. 2: Detecting Genes* (Cold Spring Harbor Laboratory Press 1998), Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics* (John Wiley & Sons 1998), and Richards and Ward, "Molecular Diagnostic Testing," in *Principles of Molecular Medicine*, pages 83-88 (Humana Press, Inc. 1998)). Direct analysis of an zmse1 gene for a mutation can be performed using a subject's genomic DNA. Methods for amplifying genomic DNA, obtained for example from peripheral blood lymphocytes, are well-known to those of skill in the art (see, for example, Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics*, at pages 7.1.6 to 7.1.7 (John Wiley & Sons 1998)).

Mice engineered to express the zmse1 gene, referred to as "transgenic mice," and mice that exhibit a complete absence of zmse1 gene function, referred to as "knockout mice," may also be generated (Snouwaert et al., Science 257:1083, 1992; Lowell et al., Nature 366:740-42, 1993; Capecchi, M.R., Science 244: 1288-1292, 1989; Palmiter, R.D. et al. Annu Rev Genet. 20: 465-499, 1986). For example,

transgenic mice that over-express zmse1, either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether over-expression causes a phenotype. For example, over-expression of a wild-type zmse1 polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes, resulting in a phenotype that identifies a tissue in which zmse1 expression is functionally relevant and may indicate a therapeutic target for the zmse1, its agonists or antagonists. For example, a preferred transgenic mouse to engineer is one that over-expresses the full length human zmse1 polypeptide (residue 1 (Met) to residue 356 (Val) of SEQ ID NO:2); or more preferably the full length mouse zmse1 polypeptide (residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5). Preferred tissue-specific or tissue-restricted promoters include lymphoid-restricted, epithelial-specific, colon-specific, ovary-specific and skin-restricted promoters. Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout zmse1 mice can be used to determine where zmse1 is absolutely required *in vivo*. A transgenic mouse that is a knockout mouse would not expresses residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5, because they would exhibit a complete absence of endogenous zmse1 gene function. The phenotype of knockout mice is predictive of the *in vivo* effects of that a zmse1 antagonist, such as those described herein, may have. The murine zmse1 mRNA, and cDNA is isolated (SEQ ID NO:4) and can be used to isolate mouse zmse1 genomic DNA (Genbank Accession No. AC026091) , which are subsequently used to generate knockout mice. These transgenic and knockout mice may be employed to study the zmse1 gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human or animal diseases (such as those in commercially viable animal populations). The mouse models of the present invention are particularly relevant as tumor models for the study of cancer biology and progression. Such models are useful in the development and efficacy of therapeutic molecules used in human cancers. Because increases in zmse1 expression, as well as decreases in zmse1 expression are associated with specific human cancers, both transgenic mice and knockout mice would serve as useful animal models for cancer. Moreover, in a preferred embodiment, zmse1 transgenic mouse can serve as an animal model for specific tumors, particularly colon cancer, ovarian cancer, leukemia or

melanoma. Moreover, transgenic mice expression of zmse1 antisense polynucleotides or ribozymes directed against zmse1, described herein, can be used analogously to transgenic mice described above.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Isolation and Cloning of the Human Zmse1

A. Using an EST Sequence to Obtain Full-length human zmse1

Scanning of a translated human cDNA database resulted in identification of an expressed sequence tag (EST) sequence which was used to identify a human full length cDNA from a K562 cDNA library prepared in house (K562 cells; ATCC No. CCL-243).

Confirmation of the full length human cDNA sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA was contained in a plasmid. The human zmse1 cDNA clone was sequenced using the following primers: ZC18489 (SEQ ID NO:21) , ZC18106 (SEQ ID NO:22), ZC18438 (SEQ ID NO:23), ZC18165 (SEQ ID NO:24), ZC18214 (SEQ ID NO:25), ZC18275 (SEQ ID NO:26), ZC18213 (SEQ ID NO:27), ZC18285 (SEQ ID NO:28), ZC18388 (SEQ ID NO:29), ZC18105 (SEQ ID NO:30), ZC18452 (SEQ ID NO:31), and vector primers ZC6,768 (SEQ ID NO:17), and ZC694. (SEQ ID NO:18). Sequencing results indicated a 3076 bp insert with a 1068 bp open reading frame beginning with an initiating Met and ending with a stop signal. The sequence analyses revealed that the cDNA encompassed the entire coding region of the DNA encoding human zmse1. The cDNA sequence is shown in SEQ ID NO:1 and the corresponding deduced polypeptide sequence is shown in SEQ ID NO:2.

Example 2

Tissue Distribution of zmse1 in Human Tissues

A. Human tissue blots probed with a human zmse1 probe

Northern blot analysis was performed using Human Multiple Tissue Blots (MTN I, MTN II, and MTN III) (Clontech, Palo Alto, Ca). A full length human zmse1 probe, based directly on the zmse1 cDNA (Example 1) was generated by PCR. The PCR fragment was gel purified using QIAquick gel extraction kit (Qiagen, Santa Clarita, Ca.). The probe was radioactively labeled with ^{32}P using the Rediprime II DNA Labeling system (Amersham, UK) according to Manufacturer's specifications. The probe was purified using a Nuctrap push column (Stratagene cloning system, La Jolla, Ca). Expresshyb (Clontech) solution was used for the hybridizing solution for the blots. Hybridization took place overnight at 65°C. The blots were then washed 4X in 2X SCC and 0.05% SDS at RT, followed by two washes in 0.1X SSC and 0.1% SDS at 50°C. One transcript size was detected at approximately 3.6 kb. Signal intensity was ubiquitous for those tissues tested.

A Dot Blot was also performed using Human RNA Master Blots™ (Clontech). The methods and conditions for the Dot Blot were the same as for the Multiple Tissue Blots disclosed above. Again, signal intensity was ubiquitous for those tissues tested.

Example 3

Chromosomal Assignment and Placement of Human Zmse1

Zmse1 was mapped to human chromosome 17 using the commercially available version of the "Stanford G3 Radiation Hybrid Mapping Panel" (Research Genetics, Inc., Huntsville, AL). The "Stanford G3 RH Panel" contains DNAs from each of 83 radiation hybrid clones of the whole human genome, plus two control DNAs (the RM donor and the A3 recipient). A publicly available WWW server (http://shgc-www.stanford.edu) allows chromosomal localization of markers.

For the mapping of Zmse1 with the "Stanford G3 RH Panel", 20 µl reactions were set up in a 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 85 PCR reactions

consisted of 2 µl 10X KlenTaq PCR reaction buffer (CLONTECH Laboratories, Inc., Palo Alto, CA), 1.6 µl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 µl sense primer, ZC18,859 (SEQ ID NO:7), 1 µl antisense primer, ZC18,860 (SEQ ID NO:8), 2 µl "RediLoad" (Research Genetics, Inc., Huntsville, AL), 0.4 µl 50X Advantage KlenTaq Polymerase Mix (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and ddH2O for a total volume of 20 µl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 94°C, 35 cycles of a 45 seconds denaturation at 94°C, 45 seconds annealing at 66°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel.

The results showed linkage of Zmse1 to the human chromosome 17 framework marker SHGC-11717 with a LOD score of 15.36 and at a distance of 8.98 cR_10000 from the marker. The use of surrounding genes/markers positions Zmse1 in the 17q24.3-q25 chromosomal region.

Example 4

Isolation and Cloning of Murine zmse1

Extension of EST Sequence

Scanning of a translated DNA database using a protein sequence consisting of the translated open reading frame of human zmse1 as a query resulted in identification of EST1166173, a murine expressed sequence tag (EST) found to be an ortholog of the human zmse1 (Example 1) (SEQ ID NO:1; SEQ ID NO:2). The mouse ortholog was designated muzmse1.

Confirmation of the EST sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA was contained in a plasmid. The mouse zmse1 cDNA clone was sequenced using the following primers: ZC19,115 (SEQ ID NO:9) ,ZC19,119 (SEQ ID NO:10), ZC19,190 (SEQ ID NO:11), ZC19,191 (SEQ ID NO:12), ZC19,192 (SEQ ID NO:13), ZC19,193 (SEQ ID NO:14), ZC19,278 (SEQ ID NO:15), ZC19,270 (SEQ ID NO:16), and vector primers ZC6,768 (SEQ ID NO:17), and ZC694. (SEQ ID NO:18). Sequencing results indicated a 2925 bp insert

with a 1050 bp open reading frame beginning with an initiating Met and ending with a stop signal. The sequence analyses revealed that the cDNA encompassed the entire coding region of the DNA encoding muzmse1. The cDNA sequence is shown in SEQ ID NO:4 and the corresponding deduced polypeptide sequence is shown in SEQ ID NO:5.

Example 5

Generation of Untagged zmse1 Recombinant Adenovirus

A. Preparation of DNA construct for generation of Adenovirus

The protein coding region of murine zmse1 was used to generate recombinant adenovirus. The 1050 bp mouse zmse1 cDNA was released from the TG12-8 vector (Example 6) using FseI and AscI enzymes. The cDNA was isolated on a 1% low melt SeaPlaque GTG™ (FMC, Rockland, ME) gel and was then excised from the gel and the gel slice melted at 70°C, extracted twice with an equal volume of Tris buffered phenol, and EtOH precipitated. The DNA was resuspended in 10 µl H_2O.

The zmse1 cDNA was cloned into the FseI-AscI sites of pAdTrack CMV (He, T-C. et al., PNAS 95:2509-2514, 1998) in which the native polylinker was replaced with FseI, EcoRV, and AscI sites. Ligation was performed using the Fast-Link™ DNA ligation and screening kit (Epicentre Technologies, Madison, WI). In order to linearize the plasmid, approximately 5 µg of the pAdTrack™ CMV mouse zmse1 plasmid was digested with PmeI. Approximately 1 µg of the linearized plasmid was cotransformed with 200 ng of supercoiled pAdEasy™ (He et al., supra.) into BJ5183 cells. The co-transformation was done using a Bio-Rad Gene Pulser at 2.5kV, 200 ohms and 25 µF. The entire co-transformation was plated on 4 LB plates containing 25 µg/ml kanamycin. The smallest colonies were picked and expanded in LB/kanamycin and recombinant adenovirus DNA identified by standard DNA miniprep procedures. Digestion of the recombinant adenovirus DNA with FseI-AscI confirmed the presence of zmse1. The recombinant adenovirus miniprep DNA was transformed into DH10B competent cells and DNA prepared using a Qiagen maxi prep kit as per kit instructions.

B. Transfection of 293A Cells with Recombinant DNA

Approximately 5 µg of recombinant adenoviral DNA was digested with PacI enzyme (New England Biolabs) for 3 hours at 37°C in a reaction volume of 100 µl containing 20-30U of PacI. The digested DNA was extracted twice with an equal volume of phenol/chloroform and precipitated with ethanol. The DNA pellet was resuspended in 10 µl distilled water. A T25 flask of QBI-293A cells (Quantum Biotechnologies, Inc. Montreal, Qc. Canada), inoculated the day before and grown to 60-70% confluence, were transfected with the PacI digested DNA. The PacI-digested DNA was diluted up to a total volume of 50 µl with sterile HBS (150mM NaCl, 20mM HEPES). In a separate tube, 20 µl DOTAP (Boerhinger Mannheim, 1 mg/ml) was diluted to a total volume of 100 µl with HBS. The DNA was added to the DOTAP, mixed gently by pipeting up and down, and left at room temperature for 15 minutes. The media was removed from the 293A cells and washed with 5 ml serum-free MEMalpha (Gibco BRL) containing 1mM Sodium Pyruvate (GibcoBRL), 0.1 mM MEM non-essential amino acids (GibcoBRL) and 25mM HEPES buffer (GibcoBRL). 5 ml of serum-free MEM was added to the 293A cells and held at 37°C . The DNA/lipid mixture was added drop-wise to the T25 flask of 293A cells, mixed gently and incubated at 37°C for 4 hours. After 4 h the media containing the DNA/lipid mixture was aspirated off and replaced with 5 ml complete MEM containing 5% fetal bovine serum. The transfected cells were monitored for Green Fluorescent Protein (GFP) expression and formation of foci, i.e., viral plaques.

Seven days after transfection of 293A cells with the recombinant adenoviral DNA, the cells expressed the GFP protein and started to form foci. These foci are viral "plaques" and the crude viral lysate was collected by using a cell scraper to detach all of the 293A cells. The lysate was transferred to a 50 ml conical tube. To release most of the virus particles from the cells, three freeze/thaw cycles were done in a dry ice/ethanol bath and a 37° water bath.

C. Amplification of Recombinant Adenovirus (rAdV)

The crude lysate was amplified (Primary (1°) amplification) to obtain a working "stock" of zmse1 rAdV lysate. Ten 10cm plates of nearly confluent (80-90%)

293A cells were set up 20 hours previously, 200 µl of crude rAdV lysate added to each 10cm plate and monitored for 48 to 72 hours looking for CPE under the white light microscope and expression of GFP under the fluorescent microscope. When all of the 293A cells showed CPE (Cytopathic Effect) this 1° stock lysate was collected and freeze/thaw cycles performed as described under Crude rAdV Lysate.

Secondary (2°) Amplification of zmse1 rAdV was obtained as follows: Twenty 15cm tissue culture dishes of 293A cells were prepared so that the cells were 80-90% confluent. All but 20 mls of 5%MEM media was removed and each dish was inoculated with 300-500 µl 1° amplified rAdv lysate. After 48 hours the 293A cells were lysed from virus production and this lysate was collected into 250 ml polypropylene centrifuge bottles and the rAdV purified.

D. AdV/eDNA Purification

NP-40 detergent was added to a final concentration of 0.5% to the bottles of crude lysate in order to lyse all cells. Bottles were placed on a rotating platform for 10 min. agitating as fast as possible without the bottles falling over. The debris was pelleted by centrifugation at 20,000 X G for 15 minutes. The supernatant was transferred to 250 ml polycarbonate centrifuge bottles and 0.5 volumes of 20%PEG8000/2.5M NaCl solution added. The bottles were shaken overnight on ice. The bottles were centrifuged at 20,000 X G for 15 minutes and supernatant discarded into a bleach solution. The white precipitate in two vertical lines along the wall of the bottle on either side of the spin mark is the precipitated virus/PEG. Using a sterile cell scraper, the precipitate from 2 bottles was resuspended in 2.5 ml PBS. The virus solution was placed in 2 ml microcentrifuge tubes and centrifuged at 14,000 X G in the microfuge for 10 minutes to remove any additional cell debris. The supernatant from the 2 ml microcentrifuge tubes was transferred into a 15 ml polypropylene snapcap tube and adjusted to a density of 1.34 g/ml with cesium chloride (CsCl). The volume of the virus solution was estimated and 0.55 g/ml of CsCl added. The CsCl was dissolved and 1 ml of this solution weighed 1.34 g. The solution was transferred polycarbonate thick-walled centrifuge tubes 3.2 ml (Beckman #362305) and spin at 80,000 rpm (348,000 X G) for 3-4 hours at 25°C in a Beckman Optima TLX microultracentrifuge

with the TLA-100.4 rotor. The virus formed a white band. Using wide-bore pipette tips, the virus band was collected.

The virus from the gradient has a large amount of CsCl which must be removed before it can be used on cells. Pharmacia PD-10 columns prepacked with Sephadex G-25M (Pharmacia) were used to desalt the virus preparation. The column was equilibrated with 20 ml of PBS. The virus was loaded and allowed it to run into the column. 5 ml of PBS was added to the column and fractions of 8-10 drops collected. The optical densities of 1:50 dilutions of each fraction was determined at 260 nm on a spectrophotometer. A clear absorbance peak was present between fractions 7-12. These fractions were pooled and the optical density (OD) of a 1:25 dilution determined. A formula is used to convert OD into virus concentration: (OD at 260nm)(25)(1.1 x 10^{12}) = virions/ml. The OD of a 1:25 dilution of the zmse1 rAdV was 0.164, giving a virus concentration of 4.5 X 10^{12} virions/ml.

To store the virus, glycerol was added to the purified virus to a final concentration of 15%, mixed gently but effectively, and stored in aliquots at -80°C .

E. Tissue Culture Infectious Dose at 50% CPE (TCID 50) Viral Titration Assay

A protocol developed by Quantum Biotechnologies, Inc. (Montreal, Qc. Canada) was followed to measure recombinant virus infectivity. Briefly, two 96-well tissue culture plates were seeded with $1X10^{4}$ 293A cells per well in MEM containing 2% fetal bovine serum for each recombinant virus to be assayed. After 24 hours 10-fold dilutions of each virus from $1X10^{-2}$ to $1X10^{-14}$ were made in MEM containing 2% fetal bovine serum. 100 µl of each dilution was placed in each of 20 wells. After 5 days at 37°C , wells were read either positive or negative for Cytopathic Effect (CPE) and a value for "Plaque Forming Units/ml" (PFU) is calculated.

$TCID_{50}$ formulation used was as per Quantum Biotechnologies, Inc., above. The titer (T) is determined from a plate where virus used is diluted from 10^{-2} to 10^{-14}, and read 5 days after the infection. At each dilution a ratio (R) of positive wells for CPE per the total number of wells is determined.

To Calculate titer of the undiluted virus sample: the factor, "F" = 1+d(S-0.5); where "S" is the sum of the ratios (R); and "d" is Log10 of the dilution series, for example, "d" is equal to 1 for a ten-fold dilution series. The titer of the undiluted sample is $T = 10^{(1+F)}$ = $TCID_{50}$/ml. To convert $TCID_{50}$/ml to pfu/ml, 0.7 is subtracted from the exponent in the calculation for titer (T).

The murine zmse1 adenovirus had a titer of 7.1×10^{10} pfu/ml.

Example 6

Generation of Construct for Transgenic Expression of Mouse zmse1 (muzmse1)

Oligonucleotides were designed to generate a PCR fragment containing a consensus Kozak sequence and the mouse zmse1 coding region. These oligonucleotides were designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into pTG12-8, our standard transgenic vector. PMT12-8 contains the mouse MT-1 promoter and a 5' rat insulin II intron upstream of the FseI site.

PCR reactions were carried out with 200 ng mouse zmse1 template (Example 4) and oligonucleotides ZC19,514 (SEQ ID NO:19) and ZC19,515 (SEQ ID NO:20). PCR reaction conditions were as follows: one cycle at 95°C for 5 minutes; followed by 15 cycles at 95°C for 1 min., 58°C for 1 min., and 72°C for 1.0 min.; followed by 72°C for 7 min.; followed by a 4°C soak. PCR products were separated by agarose gel electrophoresis and purified using a QiaQuick™ (Qiagen) gel extraction kit. The isolated, 1050 bp, DNA fragment was digested with FseI and AscI (Boerhinger-Mannheim), phenol/chloroform extracted, ethanol precipitated, resuspended in TE, and ligated into pTG12-8 that was previously digested with FseI and AscI. The pTG12-8 plasmid, designed for expression of a gene of interest in transgenic mice, contains an expression cassette flanked by 10 kb of MT-1 5' DNA and 7 kb of MT-1 3' DNA. The expression cassette comprises the MT-1 promoter, the rat insulin II intron, a polylinker for the insertion of the desired clone, and the human growth hormone poly A sequence.

About one microliter of the ligation reaction was electroporated into DH10B ElectroMax™ competent cells (GIBCO BRL, Gaithersburg, MD) according to

manufacturer's direction and plated onto LB plates containing 100 μg/ml ampicillin, and incubated overnight. Colonies were picked and grown in LB media containing 100 μg/ml ampicillin. Miniprep DNA was prepared from the picked clones and screened for the zmse1 insert by restriction digestion with EcoRI, and subsequent agarose gel electrophoresis. Maxipreps of the correct pTG-zmse1 construct were performed. A positive clone was sequenced to verify that the sequence was correct. A SalI fragment containing with 5' and 3' flanking sequences, the MT-1 promoter, the rat insulin II intron, zmse1 cDNA and the human growth hormone poly A sequence are prepared and to be used for microinjection into fertilized murine oocytes.

Qiagen Maxi Prep protocol (Qiagen) was used as per manufacturer's instruction to generate mumse1 DNA to use for further subcloning, for example, into adenovirus vectors described above (Example 5).

Example 7

Expression of zmse1 in Cancer Tissues Using NCI60 Cancer Microarray

A. Determination of genes having correlated expression with zmse1

Gene expression profile information for zmse1 was obtained from oligonucleotide and cDNA microarrays. Microarrays show the mRNA expression level of a large number of genes across a large number of cell types or cells exposed to various conditions, or cells in various replication steps, depending on the experiment. Because all of the information for all of the genes on any given microarray is obtained from the same biological experiment, and all biological experiments employing the same microarray provide results on the same set of genes, it is possible to compare the mRNA expression patterns of different genes to each other, as well as the expression pattern of a given gene in various tissues, cell lines, or cancers.

Briefly, microarray experiments are conducted by extracting the mRNA from reference tissues(s) or cell line(s) and from experimental sample tissue(s) or cell line(s). The reference mRNA is reverse transcribed to cDNA in a reaction along with a fluorescent dye label. The sample mRNA is likewise reverse transcribed to cDNA, but in the presence of a dye label with a different emission wavelength from the reference. The two cDNA samples are then mixed and hybridized to the microarray. The

microarray itself has thousands of unlabeled cDNA clones covalently bound as spots (also called 'features') on its surface. The labeled cDNAs then bind to their respective microarray spots. If a particular gene is transcribed at a higher level in the experimental sample relative to the reference, then the spot will fluoresce to a greater degree in the experimental sample dye wavelength channel. Conversely, if the gene in the experimental sample is down regulated, then the wavelength channel of the reference dye will be stronger. Finally, the microarrays are scanned at the wavelengths of both dyes and the results for each spot are recorded and stored electronically. Large numbers of microarray experiments are typically done together using the same reference cDNA, but varying the experimental conditions, cell lines, tissues, time points, and the like.

Raw and/or processed microarray expression information was obtained from a subscription data set that was electronically downloaded. Publicly available, purchased, or in-house custom designed software can be used to analyze the microarray data (E.g., the publicly available NCI60 Cancer Microarray Project (Stanford University, Palo Alto, CA) world-wide-web resource http://genome-www.stanford/nci60/search.shtml). Prior to analysis, spots were examined to exclude experimental artifacts (dust spots, substrate imperfections, incomplete or uneven hybridization washes, etc.) and absorbence was adjusted to take into account background fluorescence of the microarray substrate at both wavelengths. Very weak and very strong signals beyond the linear range response of the microarray reader were likewise excluded from analysis. Analyses were typically done on the ratio of the absorbance intensities of the reference and sample wavelength channels for each spot. These absorbance ratios were normalized to log base 2. Microarray information for zmse1 was found in Ross et al. using a 'NCI60' microarray (Ross, DT et al., Nature Genet. 24:227-235, 2000). The reference mRNA was composed of a mixture of equal quantities of mRNA from HL-60, K562, NCI-H226, COLO 205, SNB-19, LOX-IMVI, OVCAR-3, OVCAR-4, CAKI-1, PC-3, MCF7, and Hs578T cells. See, Ross et al. supra. for details of this method.

A cDNA clone corresponding to the 3' end of zmse1 cDNA (and corresponding mRNA was included on the 'NCI60' microarray chip set (Ross et al. supra.). The zmse1 cDNA clone (IMAGE clone 486682; Incyte Pharmaceuticals, Palo

Alto, CA; Genbank Accession No.'s AA044169 and AA044269) corresponds to zmse1 nucleotide positions 2435 to 3076 of SEQ ID NO:1. This chip set contained 9702 additional cloned cDNAs. Ross et al. performed 68 hybridization experiments with this chip set against 60 cancer cell lines. The data from the NCI60 microarray was purchased through the SUTECH™ Microarray Expression Database Subscription Program from Stanford Sequencing and Technology Center's Technology Development Group, (Stanford University, Palo Alto, CA) (http://otl.stanford.edu/tech/sutech.html).

Analysis was done by obtaining the Pearson correlation (R) between all pairs of spots in the entire set of microarray experiments. The Pearson correlation comprises a value from 1 to -1. A value of 1 shows that the expression in the two compared spots are positively correlated (both either are increased or decreased). A value of -1 shows that the expression in the two compared spots are negatively correlated (when one goes up, the other goes down, or visa versa). A value of 0 shows that the items are not correlated over the range of experiments. Although values between 0 and 1, or 0 and -1 can be considered positively or negatively correlated respectively, in the current analysis, correlations greater than 0.5 or less than -0.5 were considered to be significant. A similar analysis was performed by Ross et al, supra. Their results were also queried electronically by the NCI60 Cancer Microarray Project (Stanford University, Palo Alto, CA) world-wide-web resource (http://genome-www.stanford/nci60/search.shtml). Thus genes potentially co-regulated or coexpressed with zmse1 were evaluated.

Table 5 shows the results of correlated cDNA clones in the NCI60 microarray that have a Pearson's R correlation of expression greater than 0.5 or less than -0.5 with a zmse1 expression. Expressed genes are indexed by their accession number, and the corresponding protein, if known, is described.

Our analysis of the data showed that zmse1 had correlated expression (Pearson's R < -0.5 or > 0.5) with 39 other cDNA clones (Table 5). The clones having correlated expression with zmse1 included cytoskeletal, cell cycle control, and other genes. For example, Zmse1 was coexpressed with epithelial cytoskeletal proteins such as paxillin, uroplakin, and zonaula occludens protein. It also showed co-expression with the nucleotide metabolism gene, cytosolic hydroxymethyltransferase, and a

negative correlation with adenylosuccinate lyase. Examination of the results obtained by our analysis, in conjunction with the results obtained from Stanford Genomic Resources (Stanford University, Palo Alto, CA) (http://genome-www.stanford.edu/) reveals that zmse1 is coexpressed with several cytoskeletal and cell-junction genes: cytoskeletal protein (HCYT), tight junction (zonula occludens) protein ZO-1, LIM domain protein (CLP), syndecan-1, SH3 binding protein, amphiglycan, and paxillin. These results showing that zmse1 is co-expressed with cytoskeletal proteins strongly reinforces that zmse1 is involved in cytoskeletal organization as described herein. Additionally, zmse1 expression is correlated with cytosolic serine hydroxymethyltransferase and cdc2L1, and anti-correlated with adenylosuccinate lyase, DNA-directed polymerase II, and cdc25A. These results likewise suggest that zmse1 has a role in cell cycle control and cancer.

Table 5

Genbank Accession No.	Pearson's R	Description
W46185	0.623538	unknown
W30779-N94432	0.621390	cytoskeletal protein (HCYT)
R79559-R79560	0.592573	tight junction (zonula occludens) protein ZO-1
AA053648-AA053259	0.557682	cytosolic serine hydroxymethyltransferase
N47464-N47465	0.549652	unknown
R99701-R99596	0.545722	unknown
W81425	0.543336	LIM domain protein (CLP)
R01486-R00830	0.532809	syndecan-1
W94188-W74616	0.524556	breast tumor-associated protein
AA031793-AA031660	0.524266	serine/threonine kinase, NEK4a
AA024925-AA024819	0.522498	protein tyrosine phosphatase, LAR
T39472-T40608	0.519872	SH3 binding protein
R53149-R53062	0.518507	unknown
AA043212-AA043213	0.517950	HREV107-like protein
R09663-R09550	0.512873	amphiglycan

N50356-N51577	0.512019	unknown
W31174-N98734	0.510772	unknown
AA047159-AA047298	0.509458	unknown
W73440-W73379	0.509449	unknown
H24396	0.507807	unknown
AA034388-AA034389	0.507472	immunogenic prostate tumor protein
AA011515-AA011682	0.504750	unknown
W44684-W44685	0.504525	maguk p55 subfamily member 3 (mpp3 protein)
AA040884-AA040885	0.503192	calcyclin
W38993-N93209	0.502568	T84 colon carcinoma cell IL-1beta regulated HSCC1
AA004976-AA004863	0.500155	Na,K-ATPase beta subunit (ATP1B)
N77727-N58359	-0.501752	RNA-associated protein-8 (RNAAP-8)
N41802-N32849	-0.508249	unknown
W95242-W95124	-0.508527	neuroblastoma apoptosis-related RNA binding protein (NAPOR-1)
W24524-N92340	-0.511351	MLC-1V/Sb isoform
AA052965	-0.511973	bone marrow protein BM034
AA057262-AA058707	-0.513009	unknown
AA043037-AA042937	-0.513606	unknown
H59306-H59260	-0.518568	cdc25A
N80399-N67978	-0.527630	unknown
W79319-W79419	-0.533025	DNA-directed polymerase II
AA010077	-0.542907	unknown
H99588	-0.548033	lymphoid nuclear protein (LAF-4)
W92381-W92325	-0.573708	adenylosuccinate lyase (ADSL)

B. Determination of zmse1 expression in cell and tissue types, and cancers.

Zmse1 expression in the microarray hybridization data described above was also analyzed for expression in various tissue types and cancers. Table 6 shows the

Ratio of expression of zmse1 relative to the reference standard. The ratio of expression is another was to view the data. For each spot on the microarray, the ratio of fluorescence of the reference and sample wavelengths is a measure of the level of induction or repression of the test sample relative to the control (Ratio = [sample fluorescence/control reference fluorescence]). If there is no change in mRNA expression level of a given gene in the control and test samples, then the ratio for the corresponding spot will be 1. If the sample expression is induced in the test sample then the ratio of fluorescence for that spot will be greater than 1; if it is repressed then the ratio will be less than 1. The results indicated that zmse1 is up-regulated in colon cancer cell lines, and ovarian cancer cell lines. This data also indicated a down-regulation of zmse1 in leukemia and melanoma cancer cell lines. Prostate, CNS, renal, breast, and non small cell lung cancer cell lines generally showed mixed or weak changes in zmse1 expression relative to the control level. Zmse1 expression was highest in the LOX-IMVI (melanoma cell line), HOP-92 (non-small cell lung carcinoma cell line), BC2 (clinical sample of a lymph node metastasis of breast cancer), and COLO205 (colon cancer cell line). Zmse1 expression was lowest in the CCRF-CEM, RPMI-8226, MOLT-4 (leukemia cell lines), and the M-14 (melanoma cell line). These results show that a zmse1 increase or decrease in expression is correlated with certain human cancers. As such, detection of zmse1 expression increase or decrease can be used as a diagnostic for human cancers. Moreover, in a preferred embodiment, zmse1 can serve as a marker for certain tissue-specific tumors particularly colon cancer, ovarian cancer, leukemia or melanoma. Use of polynucleotides, polypeptides, and antibodies of the present invention for such diagnostic purposes are known in the art, and disclosed herein.

Table 6

Cell Line or Tissue	Description	Ratio of Zmse1 expression to reference
HS_578T	breast cancer cell line	0.51
MDA-N	breast cancer cell line	0.59
MDA-MB-435	breast cancer cell line	0.63

BT-549	breast cancer cell line	0.8
MDA-MB-231	breast cancer cell line	0.96
MCF7	breast cancer cell line	1.15
T-47D	breast cancer cell line	1.57
MCF7	breast cancer cell line	0.78
MCF7	breast cancer cell line	1.13
BC2	breast cancer lymph node metastasis	1
BC16	breast cancer tissue biopsy	1.08
BC2	breast cancer tissue biopsy	2.47
Normal Breast	breast tissue biopsy, normal	1.57
SF-539	CNS cancer cell line	0.7
SF-295	CNS cancer cell line	1
SF-268	CNS cancer cell line	1.03
SNB-19	CNS cancer cell line	1.15
U251	CNS cancer cell line	1.22
SW-620	colon cancer cell line	0.83
HCT-15	colon cancer cell line	1.02
KM12	colon cancer cell line	1.19
HCT-116	colon cancer cell line	1.38
HCC-2998	colon cancer cell line	1.42
COLO205	colon cancer cell line	3.1
HT-29	colon cancer cell line	1.13
MOLT-4	leukemia cell line	0.16
RPMI-8226	leukemia cell line	0.35
CCRF-CEM	leukemia cell line	0.43
HL-60	leukemia cell line	0.52
K-562	leukemia cell line	0.53
SR	leukemia cell line	0.98
K562	leukemia cell line	0.61

K562	leukemia cell line	0.62
M-14	melanoma cell line	0.27
SK-MEL-2	melanoma cell line	0.62
SK-MEL-5	melanoma cell line	0.7
SK-MEL-28	melanoma cell line	0.71
UACC-257	melanoma cell line	0.71
UACC-62	melanoma cell line	0.77
MALME-3M	melanoma cell line	0.89
LOXIMVI	melanoma cell line	1.86
NCI-H23	non-small cell lung cancer (NSCLC) cell line	0.53
NCI-H322	NSCLC cell line	0.69
EKVX	NSCLC cell line	0.74
NCI-H522	NSCLC cell line	0.82
NCI-H460	NSCLC cell line	1
A549	NSCLC cell line	1.08
HOP-62	NSCLC cell line	1.12
NCI-H226	NSCLC cell line	1.29
HOP-92	NSCLC cell line	2.31
OVCAR-4	ovarian cancer cell line	0.73
OVCAR-3	ovarian cancer cell line	1.04
OVCAR-8	ovarian cancer cell line	1.04
OVCAR-5	ovarian cancer cell line	1.35
IGROV1	ovarian cancer cell line	1.39
SK-OV-3	ovarian cancer cell line	1.49
DU-145	prostate cancer cell line	1.1
PC-3	prostate cancer cell line	1.19
UO-31	renal cancer cell line	0.61
RXF-393	renal cancer cell line	0.81
SNB-75	renal cancer cell line	1

786-0	renal cancer cell line	1.06
SN12C	renal cancer cell line	1.06
CAKI-1	renal cancer cell line	1.09
ACHN	renal cancer cell line	1.17
A498	renal cancer cell line	1.34
TK-10	renal cancer cell line	1.38
ADR-RES	unknown	1.28

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. An isolated polynucleotide that encodes a polypeptide comprising a sequence of amino acid residues from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 147 (Ala);

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 336 (Ser);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 356 (Ser); and

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 356 (Val).

2. An isolated polynucleotide according to claim 1, wherein the polynucleotide is from the group of:

(a) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 199 to nucleotide 639;

(b) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 640 to nucleotide 1206;

(c) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 640 to nucleotide 1266; and

(d) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 199 to nucleotide 1266.

3. An isolated polynucleotide sequence according to claim 1, wherein the polynucleotide comprises nucleotide 1 to nucleotide 1068 of SEQ ID NO:3.

4. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a polypeptide as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 356 (Val); and

a transcription terminator,

wherein the promoter is operably linked to the DNA segment, and the DNA segment is operably linked to the transcription terminator.

5. An expression vector according to claim 4, further comprising a secretory signal sequence operably linked to the DNA segment.

6. A cultured cell comprising an expression vector according to claim 4, wherein the cell expresses a polypeptide encoded by the DNA segment.

7. A DNA construct encoding a fusion protein, the DNA construct comprising:

a first DNA segment encoding a polypeptide comprising a sequence of amino acid residues from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 147 (Ala);

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 336 (Ser);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 356 (Ser);

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 337 (Arg), to amino acid number 356 (Ser); and

(e) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 356 (Val); and

at least one other DNA segment encoding an additional polypeptide,

wherein the first and other DNA segments are connected in-frame; and

wherein the first and other DNA segments encode the fusion protein.

8. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA construct encoding a fusion protein according to claim 7; and

a transcription terminator,

wherein the promoter is operably linked to the DNA construct, and the DNA construct is operably linked to the transcription terminator.

9. A cultured cell comprising an expression vector according to claim 8, wherein the cell expresses a polypeptide encoded by the DNA construct.

10. A method of producing a fusion protein comprising:

culturing a cell according to claim 9; and

isolating the polypeptide produced by the cell.

11. An isolated polypeptide comprising a sequence of amino acid residues from the group of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 147 (Ala);

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 336 (Ser);

(c) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 148 (Asn), to amino acid number 356 (Ser); and

(d) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 356 (Val).

12. A method of producing a polypeptide comprising:

culturing a cell according to claim 6; and

isolating the polypeptide produced by the cell.

13. A method of producing an antibody comprising:

inoculating an animal with a polypeptide from the group of:

(a) a polypeptide according to claim 11; and

wherein the polypeptide elicits an immune response in the animal to produce the antibody; and

isolating the antibody from the animal.

14. An antibody produced by the method of claim 13, which specifically binds to a polypeptide of claim 11.

15. The antibody of claim 14, wherein the antibody is a monoclonal antibody.

16. An antibody that specifically binds to a polypeptide of claim 11.

17. A method of detecting, in a test sample, the presence of a modulator of zmse1 protein activity, comprising:

culturing a cell into which has been introduced an expression vector according to claim 4, wherein the cell expresses the zmse1 protein encoded by the DNA segment in the presence and absence of a test sample; and

comparing levels of activity of zmse1 in the presence and absence of a test sample, by a biological or biochemical assay; and

determining from the comparison, the presence of modulator of zmse1 activity in the test sample.

18. A method for detecting a genetic abnormality in a patient, comprising:

obtaining a genetic sample from a patient;

producing a first reaction product by incubating the genetic sample with a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1, under conditions wherein said polynucleotide will hybridize to complementary polynucleotide sequence;

visualizing the first reaction product; and

comparing said first reaction product to a control reaction product from a wild type patient, wherein a difference between said first reaction product and said control reaction product is indicative of a genetic abnormality in the patient.

19. A method for detecting a cancer in a patient, comprising:

obtaining a tissue or biological sample from a patient;

incubating the tissue or biological sample with an antibody that binds SEQ ID NO:2 under conditions wherein the antibody binds to its complementary polypeptide in the tissue or biological sample;

visualizing the antibody bound in the tissue or biological sample; and

comparing levels of antibody bound in the tissue or biological sample from the patient to a normal control tissue or biological sample,

wherein an increase or decrease in the level of antibody bound to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of a cancer in the patient.

20. A method for detecting a cancer in a patient, comprising:

obtaining a tissue or biological sample from a patient;

labeling a polynucleotide comprising at least 14 contiguous nucleotides of SEQ ID NO:1 or the complement of SEQ ID NO:1;

incubating the tissue or biological sample with under conditions wherein the polynucleotide will hybridize to complementary polynucleotide sequence;

visualizing the labeled polynucleotide in the tissue or biological sample; and

comparing the level of labeled polynucleotide hybridization in the tissue or biological sample from the patient to a normal control tissue or biological sample,

wherein an increase or decrease in the labeled polynucleotide hybridization to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of a cancer in the patient.

21. A transgenic mouse, wherein the mouse over-expresses residue 1 (Met) to residue 356 (Val) of SEQ ID NO:2) or residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5.

22. A transgenic mouse according to claim 21, wherein the expression of residue 1 (Met) to residue 356 (Val) of SEQ ID NO:2) or residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5 is expressed using a tissue-specific or tissue-restricted promoter.

23. A transgenic mouse according to claim 21, wherein the expression of residue 1 (Met) to residue 356 (Val) of SEQ ID NO:2) or residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5 is expressed using an epithelial-specific, colon-specific, or ovary-specific promoter.

24. A transgenic mouse according to claim 21, wherein the mouse does not expresses residue 1 (Met) to residue 349 (Val) of SEQ ID NO:5, relative to a normal mouse.

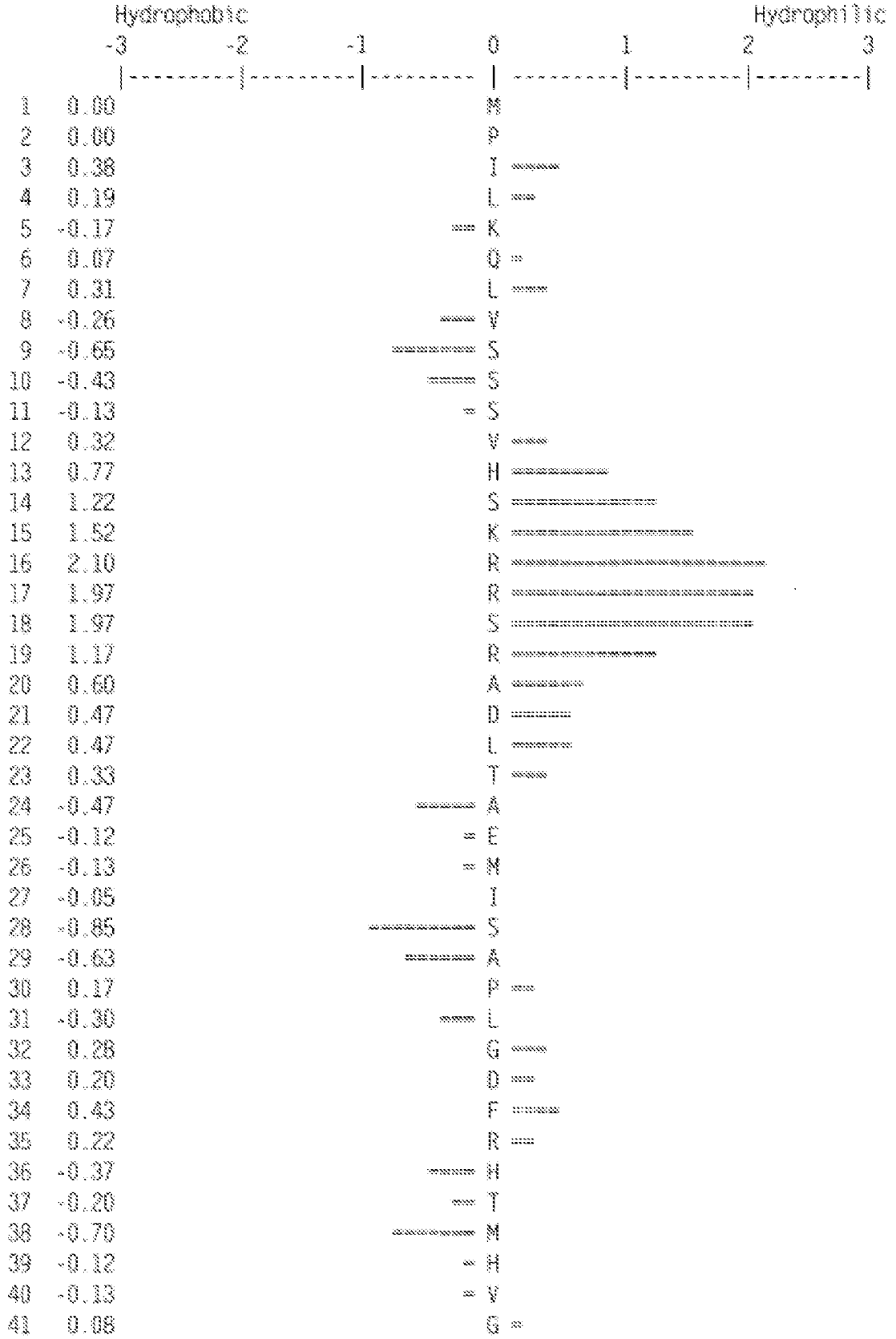
Hydrophobic
Hydrophilic
-3
-2
-1
0
1
2
3
1 0.00 M
2 0.00 P
3 0.38 I
4 0.19 L
5 -0.17 K
6 0.07 Q
7 0.31 L
8 -0.26 V
9 -0.65 S
10 -0.43 S
11 -0.13 S
12 0.32 V
13 0.77 H
14 1.22 S
15 1.52 K
16 2.10 R
17 1.97 R
18 1.97 S
19 1.17 R
20 0.60 A
21 0.47 D
22 0.47 L
23 0.33 T
24 -0.47 A
25 -0.12 E
26 -0.13 M
27 -0.05 I
28 -0.85 S
29 -0.63 A
30 0.17 P
31 -0.30 L
32 0.28 G
33 0.20 D
34 0.43 F
35 0.22 R
36 -0.37 H
37 -0.20 T
38 -0.70 M
39 -0.12 H
40 -0.13 V
41 0.08 G

FIG. 1A

42	0.67	R
43	0.83	A
44	0.42	G
45	-0.08	D
46	0.50	A
47	0.43	F
48	-0.02	G
49	-0.35	D
50	-0.23	T
51	-0.20	S
52	-0.65	F
53	-0.08	L
54	-0.22	N
55	0.20	S
56	1.00	K
57	0.97	A
58	1.42	G
59	0.92	E
60	1.50	P
61	1.55	D
62	0.75	G
63	1.25	E
64	1.25	S
65	1.28	L
66	0.78	D
67	0.78	E
68	1.13	Q
69	0.68	P
70	0.23	S
71	0.25	S
72	0.75	S
73	1.20	S
74	1.20	S
75	0.85	K
76	0.50	R
77	0.50	S
78	0.50	L
79	0.50	L
80	0.03	S
81	0.83	R
82	1.13	K
83	1.13	F
84	1.13	R
85	1.13	G

FIG. 1B

86	1.60	S
87	1.13	K
88	1.18	R
89	0.88	S
90	0.32	Q
91	0.32	S
92	0.27	V
93	0.73	T
94	1.18	R
95	1.93	G
96	2.03	E
97	2.03	R
98	2.53	E
99	1.82	Q
100	1.02	R
101	-0.05	D
102	-0.03	M
103	-0.83	L
104	-0.83	G
105	-0.12	S
106	0.23	L
107	0.72	R
108	0.37	D
109	0.25	S
110	-0.50	A
111	-0.50	L
112	-0.52	F
113	-0.52	V
114	-0.43	K
115	0.03	N
116	-0.02	A
117	-0.52	M
118	-0.52	S
119	-0.73	L
120	-0.48	P
121	-0.03	Q
122	0.77	L
123	1.27	N
124	1.15	E
125	1.37	K
126	1.83	E
127	1.83	A
128	1.33	A
129	0.77	E

FIG. 1C

130	0.90	K
131	1.48	G
132	0.68	T
133	0.18	S
134	0.68	K
135	0.80	L
136	0.45	P
137	0.00	K
138	0.35	S
139	0.40	L
140	-0.10	S
141	-0.40	S
142	0.40	S
143	0.85	P
144	0.72	V
145	0.70	K
146	1.20	K
147	1.45	A
148	1.45	N
149	0.95	D
150	1.03	G
151	1.60	E
152	1.50	G
153	2.00	G
154	1.42	D
155	1.42	E
156	1.35	E
157	1.35	A
158	1.35	G
159	0.77	T
160	0.60	E
161	0.60	E
162	1.17	A
163	1.17	V
164	0.70	P
165	0.78	R
166	0.95	R
167	0.87	N
168	0.37	G
169	-0.13	A
170	0.15	A
171	0.20	G
172	0.28	P
173	0.87	h

FIG. 1D

174	0.87	S
175	0.57	P
176	-0.05	D
177	0.40	P
178	0.90	L
179	0.43	L
180	0.35	D
181	0.23	E
182	0.53	Q
183	0.53	A
184	-0.27	F
185	-0.37	G
186	0.22	D
187	0.33	L
188	0.33	T
189	-0.42	D
190	-0.37	L
191	-0.30	P
192	-0.30	V
193	-0.08	V
194	-0.15	P
195	-0.28	K
196	-0.03	A
197	-0.33	T
198	-0.33	Y
199	-0.33	G
200	-0.35	L
201	0.53	K
202	0.58	H
203	0.58	A
204	-0.13	E
205	0.00	S
206	-0.33	I
207	-0.92	M
208	-1.27	S
209	-0.47	F
210	-0.55	H
211	-0.60	I
212	-0.18	D
213	-0.05	L
214	0.03	G
215	-0.77	P
216	-0.47	S
217	0.03	M

FIG. 1E

218	-0.22		L
219	-0.57		G
220	-0.92		D
221	-0.92		V
222	-1.13		L
223	-1.13		S
224	-0.38		I
225	0.42		K
226	1.48		D
227	1.62		K
228	2.33		E
229	1.83		E
230	1.83		W
231	1.83		D
232	1.33		P
233	2.00		E
234	1.50		E
235	1.50		G
236	1.02		E
237	0.43		G
238	0.43		G
239	0.43		Y
240	0.93		H
241	0.93		G
242	0.83		D
243	0.83		E
244	0.27		G
245	-0.30		A
246	-1.10		A
247	-1.17		G
248	-1.05		T
249	-1.05		I
250	-0.48		Y
251	-0.42		Q
252	-0.50		A
253	-0.52		P
254	-0.80		P
255	-0.80		Y
256	-0.88		A
257	-0.88		V
258	-0.50		A
259	-0.72		A
260	-0.56		P
261	0.03		P

FIG. 1F

262	0.15	L
263	0.65	A
264	0.65	R
265	1.45	Q
266	1.45	E
267	0.95	G
268	0.92	K
269	0.92	A
270	0.62	G
271	0.12	P
272	0.25	D
273	-0.05	L
274	-0.05	P
275	-0.50	S
276	-0.28	L
277	-0.37	P
278	-0.72	S
279	0.08	H
280	0.58	A
281	1.03	L
282	1.12	E
283	0.63	D
284	0.85	E
285	0.27	G
286	-0.32	W
287	-0.90	A
288	-0.90	A
289	-0.28	A
290	-0.20	A
291	-0.12	P
292	0.02	S
293	0.02	P
294	0.52	G
295	0.52	S
296	0.30	A
297	0.30	R
298	0.30	S
299	0.70	M
300	0.13	G
301	0.02	S
302	0.73	h
303	1.23	T
304	1.23	T
305	0.97	R

FIG. 1G

306	1.08		D
307	0.85		S
308	0.40		S
309	-0.67		S
310	-0.88		L
311	-1.00		S
312	-1.62		S
313	-1.32		C
314	-1.67		T
315	-1.40		S
316	-0.73		G
317	-0.17		I
318	0.90		L
319	0.95		E
320	1.25		E
321	1.47		R
322	0.55		S
323	0.55		P
324	0.05		A
325	0.00		F
326	0.50		R
327	1.08		G
328	1.42		P
329	1.42		D
330	1.33		R
331	1.25		A
332	0.50		R
333	0.05		A
334	0.63		A
335	0.17		V
336	0.25		S
337	0.83		R
338	1.58		Q
339	2.63		P
340	1.12		D
341	1.13		K
342	0.72		E
343	0.60		F
344	0.00		S
345	0.60		F
346	0.92		N
347	1.37		D
348	2.39		E

FIG. 1H

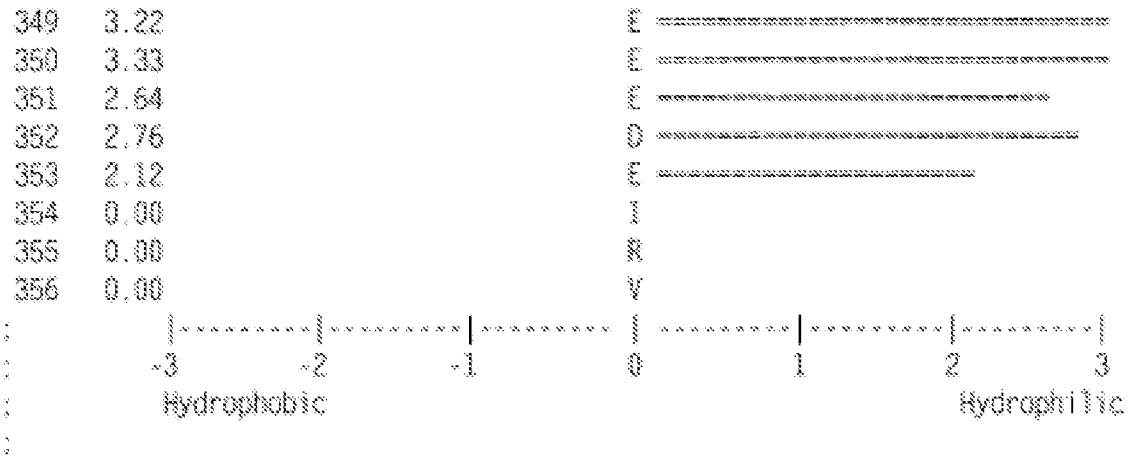
349 3.22
350 3.33
351 2.64
352 2.76
353 2.12
354 0.00
355 0.00
356 0.00
-3
-2
-1
0
1
2
3
Hydrophobic
Hydrophilic

FIG. 11

```
                10        20        30        40        50        60
MUZMSE MPILKQLVSSSVNSKRRSRADLTAEMISAPLGDFRHTMHVGRAGDAFGDTSFLTSKAREA
       ::::::::::::.:::::::::::::::::::::::::::::::::::::::::::::: :
zmsel  MPILKQLVSSSVHSKRRSRADLTAEMISAPLGDFRHTMHVGRAGDAFGDTSFLNSKAGEP
                10        20        30        40        50        60

                   70        80        90       100       110
MUZMSE QDESLDEQ--ASASKLSLLSRKFRGSKRSQSVTRGDREQRDMLGSLRDSALFVKNAMSLP
       : ::::::  .:.:: ::::::::::::::::::.:::::::::::::::::::::::::
zmsel  QGESLDEQPSSSSSKRSLLSRKFRGSKRSQSVTRGEREQRDMLGSLRDSALFVKNAMSLP
                70        80        90       100       110       120

      120       130       140       150               160       170
MUZMSE QLNEKEAAEKDSSKLPKSLSSSPVKKADARQGGPKS-------PHRNGATGPNSPDPLLD
       :::::::::: .:::::::::::::::::.  .:: ..       :.:::::.::.:::::
zmsel  QLNEKEAAEKGTSKLPKSLSSSPVKKANDGEGGDEEAGTEEAVPRRNGAAGPHSPDPLLD
               130       140       150       160       170       180

             180       190       200       210       220       230
MUZMSE EQAFGDLMDLPIMPKVSYGLKHAESILSFHIDLGPSMLGDVLSIMDKDDMGSEEEEEEAGG
       ::::::: :::.:::::::::::::::::::::::::::::::::...  :: :  ::
zmsel  EQAFGDLTDLPVVPKATYGLKHAESIMSFHIDLGPSMLGDVLSIMDKEEMDPEEGE--GG
               190       200       210       220       230

               240       250       260       270       280       290
MUZMSE YRDKEGPS-SIVQAPPVLEVVPPLGRQESKASWDQASMLPPHAVEDDGNAVVAPSPSSAR
       :.  :: . .:.::::   ..:::.:::::.:. :  : :: ::.::.::::.:::::.:::
zmsel  YHGDEGAAGTITQAPPYAVAAPPLARQEGKAGPDLPS-LPSHALEDEGNAAAAPSPGSAR
              240       250       260       270       280       290

               300       310       320       330       340
MUZMSE SVGSHTTRDSSSLSSYTSGVLEERSPAFRGPDRVAAAPPRQPDKEFCFMDEEEEDEIRV
       :.:::::::::::::: :::.:::::::::::::. ::  :::::::: ::::::::::::
zmsel  SMGSHTTRDSSSLSSCTSGILEERSPAFRGPDRARAAVSRQPDKEFSFMDEEEEDEIRV
               300       310       320       330       340       350
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FIG. 2

SEQUENCE LISTING

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<120> NOVEL CRIB PROTEIN ZMSE1

<130> 99-76PC

<150> US 09/438,564
<151> 1999-11-10

<160> 31

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 3076
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<220>
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<222> (199)...(1266)

<400> 1

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cagagctcgg gtctccgggg accgagcctt atgatctcct cattgcgtcc ccctctgccc    120
actggacttg gacttcagat ctgacccccag acctgccggc tacctcggga gggcccacct    180
ccccgcccat ccagcaag atg cca atc ctc aag caa ctg gtg tcc agc tcg        231
                  Met Pro Ile Leu Lys Gln Leu Val Ser Ser Ser
                   1               5                  10

gtg cac tcc aag cgc cgt tcc cga gcg gac ctc acg gcc gag atg atc        279
Val His Ser Lys Arg Arg Ser Arg Ala Asp Leu Thr Ala Glu Met Ile
             15                  20                  25

agc gcc ccg ctg ggc gac ttc cgc cac acc atg cac gtt ggc cgg gcc        327
Ser Ala Pro Leu Gly Asp Phe Arg His Thr Met His Val Gly Arg Ala
         30                  35                  40

gga gac gcc ttt ggg gac acc tcc ttc ctc aat agc aag gct ggc gag        375
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Gly Asp Ala Phe Gly Asp Thr Ser Phe Leu Asn Ser Lys Ala Gly Glu
     45                  50                  55

ccc gac ggc gag tcc ttg gac gaa cag ccc tct tct tca tct tcc aaa      423
Pro Asp Gly Glu Ser Leu Asp Glu Gln Pro Ser Ser Ser Ser Ser Lys
 60                  65                  70                  75

cgc agt ctc ctg tcc agg aag ttc cgg ggc agc aag cgg tca cag tcg      471
Arg Ser Leu Leu Ser Arg Lys Phe Arg Gly Ser Lys Arg Ser Gln Ser
                 80                  85                  90

gtg acc agg ggg gag cgg gag cag cgt gac atg ctg ggc tcc ctg cgg      519
Val Thr Arg Gly Glu Arg Glu Gln Arg Asp Met Leu Gly Ser Leu Arg
             95                 100                 105

gac tcg gcc ctg ttt gtc aag aat gcc atg tcc ctg ccc cag ctc aat      567
Asp Ser Ala Leu Phe Val Lys Asn Ala Met Ser Leu Pro Gln Leu Asn
        110                 115                 120

gag aag gag gcc gcg gag aag ggc acc agt aag ctg ccc aag agc ctg      615
Glu Lys Glu Ala Ala Glu Lys Gly Thr Ser Lys Leu Pro Lys Ser Leu
    125                 130                 135

tca tcc agc ccc gtg aag aag gcc aat gac ggg gag ggc ggc gat gag      663
Ser Ser Ser Pro Val Lys Lys Ala Asn Asp Gly Glu Gly Gly Asp Glu
140                 145                 150                 155

gag gcg ggc acg gag gag gca gtg ccc cgt cgg aat ggg gcc gcg ggt      711
Glu Ala Gly Thr Glu Glu Ala Val Pro Arg Arg Asn Gly Ala Ala Gly
                160                 165                 170

cca cat tcc cct gac ccc ctc ctc gat gag cag gcc ttt ggg gat ctg      759
Pro His Ser Pro Asp Pro Leu Leu Asp Glu Gln Ala Phe Gly Asp Leu
            175                 180                 185

aca gat ctg cct gtc gtg ccc aag gcc acg tac ggg ctg aag cat gcg      807
Thr Asp Leu Pro Val Val Pro Lys Ala Thr Tyr Gly Leu Lys His Ala
        190                 195                 200

gag tcc atc atg tcc ttc cac atc gac ctg ggg ccc tcc atg ctg ggt      855
Glu Ser Ile Met Ser Phe His Ile Asp Leu Gly Pro Ser Met Leu Gly
    205                 210                 215
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gac gtc ctc agc atc atg gac aag gag gag tgg gac ccc gag gag ggg      903
Asp Val Leu Ser Ile Met Asp Lys Glu Glu Trp Asp Pro Glu Glu Gly
220                 225                 230                 235

gag ggt ggt tac cat ggc gat gag ggc gcc gct ggc acc atc acc cag      951
Glu Gly Gly Tyr His Gly Asp Glu Gly Ala Ala Gly Thr Ile Thr Gln
                240                 245                 250

gct ccc ccg tac gcc gtg gcg gcc cct ccc ctg gca agg cag gaa ggc      999
Ala Pro Pro Tyr Ala Val Ala Ala Pro Pro Leu Ala Arg Gln Glu Gly
            255                 260                 265

aag gct ggc cca gac ttg ccc tcc ctc ccc tcc cat gct ctg gag gat     1047
Lys Ala Gly Pro Asp Leu Pro Ser Leu Pro Ser His Ala Leu Glu Asp
        270                 275                 280

gag ggg tgg gca gca gcg gcc ccc agc ccc ggc tca gcc cgc agc atg     1095
Glu Gly Trp Ala Ala Ala Ala Pro Ser Pro Gly Ser Ala Arg Ser Met
    285                 290                 295

ggc agc cac acc aca cgg gac agc agc tcc ctc tcc agc tgc acc tca     1143
Gly Ser His Thr Thr Arg Asp Ser Ser Ser Leu Ser Ser Cys Thr Ser
300                 305                 310                 315

ggc atc ctg gag gag cgc agc cct gcc ttc cgg ggg ccg gac agg gcc     1191
Gly Ile Leu Glu Glu Arg Ser Pro Ala Phe Arg Gly Pro Asp Arg Ala
                320                 325                 330

cgg gct gct gtc tca aga cag cca gac aag gag ttc tcc ttc atg gat     1239
Arg Ala Ala Val Ser Arg Gln Pro Asp Lys Glu Phe Ser Phe Met Asp
            335                 340                 345

gag gag gag gag gat gaa atc cgt gtg tgaggcggac agtgggtggc           1286
Glu Glu Glu Glu Asp Glu Ile Arg Val
        350                 355

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tggctgccig acttcatcat gctcccctgca cttaggctgc gtgggacaag ggctgtgttg  1526
tcacagcagg aataggtttt cctctgttgg cctcccttic ctccaccctg gcctcaaatg  1586
gatgccagat gccaacccca gttctggcca cgtacagcca gcgggtcagc ccagaggcag  1646
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ccctccctgc cccaccccag ctcctgccct ggaaatctgg agccccttgg agctgagctg   1826
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tcctgtttcc aatagcttag gtcccatggt gggggaggaa cctcaggggc tatgcagccc   2966
ccgccagctg ccctcgaatc ccgtccaggc caattccaga ttctaaactg attttttca   3026
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        35                  40                  45
Asp Thr Ser Phe Leu Asn Ser Lys Ala Gly Glu Pro Asp Gly Glu Ser
    50                  55                  60
Leu Asp Glu Gln Pro Ser Ser Ser Ser Ser Lys Arg Ser Leu Leu Ser
65                  70                  75                  80
Arg Lys Phe Arg Gly Ser Lys Arg Ser Gln Ser Val Thr Arg Gly Glu
                85                  90                  95
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Val Lys Asn Ala Met Ser Leu Pro Gln Leu Asn Glu Lys Glu Ala Ala
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Lys Lys Ala Asn Asp Gly Glu Gly Gly Asp Glu Glu Ala Gly Thr Glu
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Glu Ala Val Pro Arg Arg Asn Gly Ala Ala Gly Pro His Ser Pro Asp
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Pro Leu Leu Asp Glu Gln Ala Phe Gly Asp Leu Thr Asp Leu Pro Val
180 185 190
Val Pro Lys Ala Thr Tyr Gly Leu Lys His Ala Glu Ser Ile Met Ser
195 200 205
Phe His Ile Asp Leu Gly Pro Ser Met Leu Gly Asp Val Leu Ser Ile
210 215 220
Met Asp Lys Glu Glu Trp Asp Pro Glu Glu Gly Glu Gly Gly Tyr His
225 230 235 240
Gly Asp Glu Gly Ala Ala Gly Thr Ile Thr Gln Ala Pro Pro Tyr Ala
245 250 255
Val Ala Ala Pro Pro Leu Ala Arg Gln Glu Gly Lys Ala Gly Pro Asp
260 265 270
Leu Pro Ser Leu Pro Ser His Ala Leu Glu Asp Glu Gly Trp Ala Ala
275 280 285
Ala Ala Pro Ser Pro Gly Ser Ala Arg Ser Met Gly Ser His Thr Thr
290 295 300
Arg Asp Ser Ser Ser Leu Ser Ser Cys Thr Ser Gly Ile Leu Glu Glu
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gayggngarw snytngayga rcarccnwsn wsnwsnwsnw snaarngnws nytnytnwsn 240
mgnaarttym gnggnwsnaa rngnwsncar wsngtnacnm gnggngarng ngarcarngn 300
gayatgytng gnwsnytnmg ngaywsngcn ytnttygtna araaygcnat gwsnytnccn 360
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wsnwsnccng tnaaraargc naaygayggn garggnggng aygargargc nggnacngar 480
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aarcaygcng arwsnathat gwsnttycay athgayytng gnccnwsnat gytnggngay 660
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ccnytngcnm gncargargg naargcnggn ccngayytnc cnwsnytncc nwsncaygcn 840
ytngargayg arggntgggc ngcngcngcn ccnwsnccng gnwsngcnmg nwsnatgggn 900
wsncayacna cnngngaywс nwsnwsnytn wsnwsntgya cnwsnggnat hytngargar 960
mgnwsnccng cnttymgngg nccngaymgn gcnngngcng cngtnwsnmg ncarccngay 1020
aargarttyw snttyatgga ygargargar gargaygara thngngtn 1068

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cccatacctg cctgttgcct tgggagtgcc cagctccccc gcagccagca cg atg ccc 178
Met Pro
1

att ctc aaa cag ctg gtg tcc agc tct gtg aac tcg aag cgc cgc tca 226
Ile Leu Lys Gln Leu Val Ser Ser Ser Val Asn Ser Lys Arg Arg Ser
5 10 15

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cgt gcg gac ctc aca gcc gag atg atc agt gcc ccg ctg ggt gac ttc      274
Arg Ala Asp Leu Thr Ala Glu Met Ile Ser Ala Pro Leu Gly Asp Phe
     20                  25                  30

cgc cac acc atg cat gtg ggc cgg gct ggg gac gcc ttt ggg gac acc      322
Arg His Thr Met His Val Gly Arg Ala Gly Asp Ala Phe Gly Asp Thr
 35                  40                  45                  50

tcc ttc ctc act agc aag gcc agg gag gca gac gac gag tcc ctg gat      370
Ser Phe Leu Thr Ser Lys Ala Arg Glu Ala Asp Asp Glu Ser Leu Asp
                 55                  60                  65

gag cag gcc tcc gct tcc aag ctc agc ctc ctg tcc agg aag ttc cgg      418
Glu Gln Ala Ser Ala Ser Lys Leu Ser Leu Leu Ser Arg Lys Phe Arg
             70                  75                  80

ggc agc aaa cgt tca cag tcc gtg acc aga ggg gac cgg gag cag aga      466
Gly Ser Lys Arg Ser Gln Ser Val Thr Arg Gly Asp Arg Glu Gln Arg
         85                  90                  95

gac atg ctg ggc tcc ctg cgg gac tca gca ctg ttt gtc aag aat gcc      514
Asp Met Leu Gly Ser Leu Arg Asp Ser Ala Leu Phe Val Lys Asn Ala
    100                 105                 110

atg tcc ctg cct cag ctc aat gag aag gaa gcc gcg gag aag gac tcg      562
Met Ser Leu Pro Gln Leu Asn Glu Lys Glu Ala Ala Glu Lys Asp Ser
115                 120                 125                 130

agc aag ctg ccc aag agc ctg tcg tcc agc cct gtg aag aag gca gac      610
Ser Lys Leu Pro Lys Ser Leu Ser Ser Ser Pro Val Lys Lys Ala Asp
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gct aga gat ggt ggc ccg aag agt ccc cat cgg aac ggg gcc aca ggc      658
Ala Arg Asp Gly Gly Pro Lys Ser Pro His Arg Asn Gly Ala Thr Gly
            150                 155                 160

ccc aac tca cct gac cca ctc ctt gac gag cag gcc ttt ggg gac ctg      706
Pro Asn Ser Pro Asp Pro Leu Leu Asp Glu Gln Ala Phe Gly Asp Leu
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atg gat ctg ccc atc atg ccc aaa gtc agc tac ggg ctg aag cat gca      754
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Met Asp Leu Pro Ile Met Pro Lys Val Ser Tyr Gly Leu Lys His Ala
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gag tcg atc ctg tcc ttc cac atc gac ctg ggg cct tcc atg ctg gga      802
Glu Ser Ile Leu Ser Phe His Ile Asp Leu Gly Pro Ser Met Leu Gly
195                 200                 205                 210

gat gtt ctc agc atc atg gac aag gac cag tgg ggc tca gag gag gag      850
Asp Val Leu Ser Ile Met Asp Lys Asp Gln Trp Gly Ser Glu Glu Glu
                215                 220                 225

gag gaa gct ggc ggg tac cgt gac aag gaa ggc ccc agc agc att gtc      898
Glu Glu Ala Gly Gly Tyr Arg Asp Lys Glu Gly Pro Ser Ser Ile Val
            230                 235                 240

cag gca ccc cct gtg ctg gag gtg gtt cct cct cta ggg aga cag gaa      946
Gln Ala Pro Pro Val Leu Glu Val Val Pro Pro Leu Gly Arg Gln Glu
        245                 250                 255

agc aag gcc agc tgg gac cag gcc tct atg ctg ccc ccc cac gct gtg      994
Ser Lys Ala Ser Trp Asp Gln Ala Ser Met Leu Pro Pro His Ala Val
    260                 265                 270

gag gat gac gga tgg gcg gtg gta gcc ccc agc ccc agc tca gca cgc     1042
Glu Asp Asp Gly Trp Ala Val Val Ala Pro Ser Pro Ser Ser Ala Arg
275                 280                 285                 290

agt gtg ggc agc cac acc acg cgg gac agc agc tcc ctg tcc agc tac     1090
Ser Val Gly Ser His Thr Thr Arg Asp Ser Ser Ser Leu Ser Ser Tyr
                295                 300                 305

acc tca ggc gtc ctt gag gag cgc agc cca gct ttc aga ggc cca gac     1138
Thr Ser Gly Val Leu Glu Glu Arg Ser Pro Ala Phe Arg Gly Pro Asp
            310                 315                 320

agg gtg gca gct gct ccc cca agg cag cca gac aag gaa ttc tgc ttc     1186
Arg Val Ala Ala Ala Pro Pro Arg Gln Pro Asp Lys Glu Phe Cys Phe
        325                 330                 335

atg gat gag gag gag gaa gat gag atc cga gtt tgaggctgga ccgaaagttg   1239
Met Asp Glu Glu Glu Glu Asp Glu Ile Arg Val
    340                 345
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<400> 5

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Asp Phe Arg His Thr Met His Val Gly Arg Ala Gly Asp Ala Phe Gly
        35                  40                  45
Asp Thr Ser Phe Leu Thr Ser Lys Ala Arg Glu Ala Asp Asp Glu Ser
    50                  55                  60
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Leu Asp Glu Gln Ala Ser Ala Ser Lys Leu Ser Leu Leu Ser Arg Lys
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Phe Arg Gly Ser Lys Arg Ser Gln Ser Val Thr Arg Gly Asp Arg Glu
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Gln Arg Asp Met Leu Gly Ser Leu Arg Asp Ser Ala Leu Phe Val Lys
            100                 105                 110
Asn Ala Met Ser Leu Pro Gln Leu Asn Glu Lys Glu Ala Ala Glu Lys
        115                 120                 125
Asp Ser Ser Lys Leu Pro Lys Ser Leu Ser Ser Ser Pro Val Lys Lys
    130                 135                 140
Ala Asp Ala Arg Asp Gly Gly Pro Lys Ser Pro His Arg Asn Gly Ala
145                 150                 155                 160
Thr Gly Pro Asn Ser Pro Asp Pro Leu Leu Asp Glu Gln Ala Phe Gly
                165                 170                 175
Asp Leu Met Asp Leu Pro Ile Met Pro Lys Val Ser Tyr Gly Leu Lys
            180                 185                 190
His Ala Glu Ser Ile Leu Ser Phe His Ile Asp Leu Gly Pro Ser Met
        195                 200                 205
Leu Gly Asp Val Leu Ser Ile Met Asp Lys Asp Gln Trp Gly Ser Glu
    210                 215                 220
Glu Glu Glu Glu Ala Gly Gly Tyr Arg Asp Lys Glu Gly Pro Ser Ser
225                 230                 235                 240
Ile Val Gln Ala Pro Pro Val Leu Glu Val Val Pro Pro Leu Gly Arg
                245                 250                 255
Gln Glu Ser Lys Ala Ser Trp Asp Gln Ala Ser Met Leu Pro Pro His
            260                 265                 270
Ala Val Glu Asp Asp Gly Trp Ala Val Val Ala Pro Ser Pro Ser Ser
        275                 280                 285
Ala Arg Ser Val Gly Ser His Thr Thr Arg Asp Ser Ser Ser Leu Ser
    290                 295                 300
Ser Tyr Thr Ser Gly Val Leu Glu Glu Arg Ser Pro Ala Phe Arg Gly
305                 310                 315                 320
Pro Asp Arg Val Ala Ala Ala Pro Pro Arg Gln Pro Asp Lys Glu Phe
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<223> Consensus CRIB motif (Bubelo, PD et al.,
J.Biol.Chem. 270:29071-29074, p. 29073, 1995)

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<210> 8
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<210> 11
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<210> 17
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<400> 30
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